BY C. H. WADDINGTON

How Animals Develop
Introduction to Modern Genetics
Principles of Embryology

The Scientific Attitude
(Penguin Books)

Organisers and Genes
The Epigenetics of Birds
(Cambridge University Press)

THE STRATEGY OF THE GENES

A Discussion of Some Aspects of Theoretical Biology

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Ruskin House
GEORGE ALLEN & UNWIN LTD
MUSEUM STREET LONDON

FIRST PUBLISHED IN 1957

PRINTED IN GREAT BRITAIN
in 12 point Bembo type
BY J W ARROWSMITH LTD.
WINTERSTOKE ROAD, BRISTOL 3

PREFACE

THE FIVE essays which form the body of this book, and the sixth one which my friend Dr Henrik Kacser has kindly allowed me to include, are concerned with two of the major classical problems of biology—firstly, the nature of biological organisation and the developmental processes which bring it into being, and secondly, the theory of evolution These matters have been debated for centuries At the present time they tend, perhaps, to be somewhat thrown into the shadow by the bright light which beats on the growing points of our understanding of analytical biology But the search for the secret of gene action, for a comprehension of how enzymes operate, or of the significance of nucleic acids and *proteins in determining specificity, although exciting and of* profound importance, nevertheless do not comprise the whole aim of biology One can adequately appreciate their relevance only by reference to a framework of ideas sufficiently wide to embrace all aspects of living systems It is the purpose of Theoretical Biology—a young, and it must be confessed not as yet very substantial branch of science—to provide such a conceptual scheme These essays are offered as a contribution towards the scaffolding in one wing of the whole edifice

Part of the first essay is taken from a paper printed in the Proceedings of the Tenth International Congress of Philosophy, the second essay incorporates some paragraphs from an article which appeared in *Recent Developments in Cell Physiology* (Butterworths, 1954), part of the third is from a paper published in *Evolution, Seventh Symposium of the Society of Experimental Biology* (Cambridge University Press 1953), and a few pages of the fifth are quoted from an article published in *Endeavour* (Vol 12, July 1953) A few of the figures have been taken from published sources, to which reference is made in the captions I should like to thank the editors and publishers concerned for permission to use this material

C H W

CONTENTS

CHAPTER 1

FORM, END AND TIME

THE ENTITIES studied by physics and chemistry are commonly referred to as 'things', while the objects of biological investigation are 'organisms' Perhaps many of the biologists who have in recent years been pushing the growing points of scientific advance into ever more penetrating analysis of the operations of biological systems would be inclined to deny the reality of any distinction, or [illegible]

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rather than on the laboratory bench, the more he tends to emphasise the reality of a peculiar and special quality which distinguishes the objects of his study There would be nothing in the slightest surprising in this, and no one would have any hesitation in accepting the biologist's word for it, were it not that some authors have suggested that biological organisms are not merely distinguishable from non-living things, but are quite different in kind, involving some principle of constitution which is not elsewhere exemplified in the world

Although, in face of the enormous recent successes of che[illegible] and physics, this point of view has become very unfashiona[illegible] is by no means impossible that we shall eventually be d[illegible] to it So long as mind remains a phenomenon unme[illegible] vocabulary of the physical sciences, one can have [illegible] confidence that the stock of concepts at present [illegible] disciplines will ever be able to encompass it Such [illegible] that our fundamental scientific ideas may yet p[illegible]

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life The most useful response to the criti[illegible]

mechanistic biology is not to deny the reality of the facts to which they point, but to see how far they can be incorporated within an expanded framework of ideas derived from experimental studies

Those who argue that living organisms are fundamentally different to inanimate things usually stress three separate, though perhaps ultimately related, points of divergence Mind, Form and End I shall not attempt, in this short essay, to say anything about the first of these, but shall confine myself to the two latter, which are easier to handle in that they are undoubtedly expressed within the realm of the material

All living organisms except possibly some of the very simplest, though even that exception is doubtful are characterised by possessing a characteristic form or shape This is not a mere static configuration in which the organism happens to be produced and which it simply retains, as a stone or a mass of iron might do The form of a living plant or animal is continuously kept in being in spite of the fact that material is passing all the time through the system The form is not only, in many cases, a complex one, but the entity by which it is expressed is more nearly comparable to a river than to a mass of solid rock. Finally, organic forms have a quality, difficult to express precisely in words, but rather definitely recognisable in practice, which is often referred to as 'wholeness' or 'integration' This is almost an aesthetic quality—a character of self-sufficiency and completeness But it is not solely aesthetic It is connected with the second major peculiarity of living things, their 'directiveness', as E S Russell (1945) has called it This refers to the fact that most of the activities of a living organism are of such a kind that they tend to produce a certain characteristic end-result The most inclusive type of end-result, it is often stated, is the continued life of the organism, actually its reproduction, and the passing of its hereditary qualities to the next generation, should be regarded as a still more general goal And it is, of course, possible to define many more subsidiary goals, to which the operations of particular parts of the organism are related, for instance, the preservation of the constancy of the internal medium of the body, or many others

The use of the word 'organism' to refer to living systems is

usually intended to convey some reference to these two characteristics of Form and End. Many biologists in recent years have argued that an understanding of organisation is in Sinnott's words, 'the biological problem to which every other is subordinate and contributory' Several, such as Needham (1936) and L. J Henderson (1917), see organisation as one of the major categories 'which stand beside those of matter and energy' This seems to imply a renunciation of any attempt to formulate the problems of organisation in terms of anything else but itself It is surely premature to go so far as this until we are driven to

We can make some progress towards analysing the concepts of 'organism' or 'organisation' by starting from the consideration that these words are intended to imply some kind of coherence within the entity referred to, that is to say, they are concerned with the relation between the thing and its parts The degree of organisation is the extent to which the entity is internally coherent, in the sense that the properties of its parts are essentially dependent on the relations between the parts and the whole The relation between the part and the whole is spoken of as the function of the part

Now an entity can be divided into parts in innumerable different ways We might use the word 'organisation' to refer to the relation between the whole and the parts, when the latter were [illegible]

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it becomes senseless to speak of organisation without specifying or implying some rule by which the parts are to be discriminated Thus 'organised' must always mean 'organised when regarded from a certain point of view'

The degree of organisation of one and the same entity may be quite different according to the point of view from which it is regarded For instance, at a certain stage in the development of the eye, fragments of the eye-cup when isolated will form small eyes which are completely normal in shape, although the tissues of which they are composed are restricted to those which the fragment would have produced if left undisturbed Clearly, the state

of organisation of the eye-cup at this stage varies according as it is viewed from a morphological or from a histological standpoint

Many discussions of organisation appear to imply that, when we try to decide how far a given entity is organised, we already know the kind of parts into which it can appropriately be broken down Thus it is often suggested that, if confronted with a motor car, we must begin by considering it as an assemblage of physical pieces of metal, the cylinders, pistons, crank-shaft, wheels, etc Then the organisation of these into a patterned whole which functions as a means of locomotion appears as something additional to the collectivity of the bare pieces, and in this case the source of the additional element can be found in the design of the maker But this suggestion overlooks the fact that in many cases (particularly where the 'parts' are scientific concepts such as atoms, electrons, etc) the parts were not discovered prior to the observation of the wholes, but, on the contrary, are derived from them It is therefore, more generally important to discuss, not how already-known parts may be fitted together to give organised wholes, but how such wholes may be analysed into parts relevant to a particular point of view Thus, in considering the motor-car, it is rather artificial to state that one part of it is a conglomerate lump of a certain shape consisting of metal, mica and porcelain, and then to ask how it comes about that this functions as a sparking plug It is more natural to observe that the whole thing runs, that this necessitates a part which fires the cylinders, and thus to arrive at a definition of the sparking plug which still retains a reference to the essential function it fulfils in the running of the car

When considering a man-made system, we can discover the point of view from which it is organised by asking the maker what he had in mind when designing it If this, or something like it, were the only way of discovering the existence of organisation, any biological theory which utilised the concept would be essentially teleological Now it seems certain that, if accustomed to man-made machines, we could discover the purpose of any new one which we found, even if the maker were not available for interrogation Can one conclude from this that we can legitimately claim to recognise organisation in entities not made by man, and

therefore of a category such that for no example of the class have we been told the point of view from which they are organised?

In order that this should be possible, it is only necessary that the phenomena under observation are of such a kind that they give rise to a point of view which can be utilised to discriminate parts within the wholes whose degree of organisation is to be assessed There is no doubt that some such points of view arise without involving teleological considerations, for instance, the spatial A collection of magnets floating on the surface of a pool of water form an entity which if analysed from the geometrical point of view, turns out to be rather highly organised, since the position of any one magnet is dependent on its position within the whole The formulation of other modes of abstraction (e g the anatomical, the physiological, the behaviourist and so on) no more involves a reference outside the field of observation than does that of the spatial, but, like it, they probably depend essentially on the occurrence of a number of instances, rather than only one, to which they are relevant.

As an example of the reality of the problem of organisation, consider a case in which it is still undecided The chromosomes are found to be the bearers of hereditary determination The phenomena of crossing-over, and of chromosome rearrangements, lead to an analysis of chromosomes into spatial parts (material genes) It is then a genuine problem whether the relation between the material gene and the chromosome is determinant of the nature of the gene, evidence against this has been drawn from the comparatively slight effect of major inversions, translocations, etc, and evidence for it from the so-called position effect A definite meaning could be attached to the statement that a high degree of organisation had been discovered, and this meaning would involve no teleological reference.

There is one category of points of view which, in my opinion, is particularly characteristic of biological entities Perhaps the main respect in which the biological picture is more complex than the physical one, is the way in which time is involved in it In the Newtonian system, time was one of the elements in the physical world quite separate from any of the others, a material body of a

given mass just existed, unchanging and, indeed, quite indifferent to the passage of time But time and change is part of the essence of life Not only so, to provide anything like an adequate picture of a living thing, one has to consider it as affected by at least three different types of temporal change, all going on simultaneously and continuously

These three time-elements in the biological picture differ in scale On the largest scale is evolution, any living thing must be thought of as the product of a long line of ancestors and itself the potential ancestor of a line of descendants On the medium scale, an animal or plant must be thought of as something which has a life history It is not enough to see that horse pulling a cart past the window as the good working horse it is today, the picture must also include the minute fertilised egg, the embryo in its mother's womb, and the broken-down old nag it will eventually become Finally, on the shortest time-scale, a living thing keeps itself going only by a rapid turnover of energy or chemical change, it takes in and digests food, it breathes, and so on.

In the biological picture towards which we are finding our way, the three time systems will have to be kept in mind together That is the feat which common sense still finds difficult Even in current biology, most of our theories are still only partly formed because they leave one or other of the time scales out of account.

Let us put each of the scales in turn into the centre of the stage, and see how the other two could, if they were brought in, lead to a richer and deeper comprehension.

Taking the largest scale, we have a theory of how characters are passed on from one generation to the next—the theory of genetics It is easy to add to this the contribution of the smallest scale, whatever an animal may have inherited from its ancestors will be tested for efficient functioning in the rough and tumble of daily life These two time-scales taken together give us the Theory of Natural Selection. But to suppose that the evolution of the wonderfully adapted biological mechanisms has depended only on a selection out of a haphazard set of variations, each produced by blind chance, is like suggesting that if we went on throwing bricks together into heaps we should eventually be able

to choose ourselves the most desirable house If we bring in the medium time-scale, however, this difficulty disappears [illegible] hereditary differences which arise in animals are not [illegible] like the differences between two heaps of bricks They result [illegible] changes in orderly systems of development, and each new [illegible] has an order of its own, may be less, but sometimes more, [illegible] than that of the original from which it was derived

Again, if we start from the medium time-scale, we [illegible] series of facts about development which make up the [illegible] embryology Bringing in the large scale, we find [illegible] throws light on development—an animal during its [illegible] passes through stages which recall the development of [illegible] tors Only in the last few decades have biologists [illegible] short time-scale to the picture, and study the [illegible] processes which bring development about, but [illegible] this attempt will lead to a new depth of understanding [illegible] histories

Finally, the science which centres on the [illegible] physiology But we have to remember that the [illegible] to-day life do not just keep the animal alive in [illegible] tion. Repeating day after day, they alter slowly [illegible] and produce the medium scale changes of development [illegible] somewhere hidden among the deepest secrets of [illegible] the cell, there must be the process by which the [illegible] undergo those sudden mutations which are the [illegible] time-scale evolution.

Thus, all three time-scales are essential for the [illegible]

[illegible]

phrases into which the notes build themselves, [illegible] evolution is like the structure of the whole [illegible] in which the melodies are repeated and varied

Returning to the concepts of Form and End [illegible] earlier as properties of organisms which [illegible]

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the main because one tends to view them from an inappropriate time-scale The cause-effect sequences which we manipulate so constantly in our dealing with material things, and the great successes of physiological and biochemical studies of living organisms, both tempt us to adopt almost instinctively what I have spoken of as the short-term time scale, that appropriate to short stretches of the organism's lifetime It is from this standpoint that Form and End seem so unaccountable Is Form any more than an orderliness in the processes of development, or Goal-seeking and End more than activity consonant with the laws of Natural Selection which govern evolution? Possibly the answer is Yes, and something over and above this is involved But at least we can try how far we can go along those paths before being driven to invoke anything else

The essays which follow will not, of course, attempt to deal with more than one or two aspects of the enormously complicated and important fields of embryonic development and biological evolution. They will, indeed, leave largely on one side the two fields in which most rapid progress has recently been made, and in which, perhaps, general interest is at present most concentrated In the context of development, a whole host of investigators is at present actively attacking the fundamental problem of how a gene operates The question is being converged on from many sides by studies of the genetic control of single enzymes, by investigations on the chemical structure of the gene constituents, protein and nucleic acids, by mutation studies, analysis of pseudo-alleles and position effects, and from many other angles Results of great interest and the most scintillating promise have already been achieved, though a satisfactory solution still seems round several corners, rather than only one The experimental investigation of evolutionary processes has also notoriously made very great strides in recent years In particular the group of students of *Drosophila* associated with Dobzhansky and with the Texas school have given us a quite new outlook on the genetic constitution of wild populations and the ways in which it is organised

Both these great modern developments are mainly concerned,

however, with what I should venture to call tactical questions They aim at elucidating the way in which individual genes operate during development, or the nature of the genetic differences between very nearly related populations which may or may not be engaged in any major evolutionary advance I wish to discuss the strategic question how does development produce entities which have Form, in the sense of integration or wholeness, how does evolution bring into being organisms which have Ends, in the sense of goal seeking or directiveness?

Now admittedly the carrying out of any strategy depends on the technical means available and even the strategic conception may be limited by them But that does not mean that one cannot usefully discuss strategic matters until one has mastered every detail of the tactical methods which can be employed It is not necessary, in fact, to await a full understanding of the chemistry of single genes before trying to form some theoretical picture of how gene-systems produce integrated patterns of developmental change And to defer a discussion of the fundamental problems of evolution until we have a complete knowledge of the population genetics of local variation would be no more sensible than to postpone the study of the major religions of mankind until we comprehended such sociological minutiae as the reasons why Frenchmen can wear more sparkling shoes and Americans flashier ties than those which would be 'fittest to survive' in Great Britain It may even be that the answers to the most basic tactical questions are not merely inessential, but are actually more or less irrelevant, to the strategic problems Suppose, for instance, that we felt justified in assuming that one of the general mechanisms which could be invoked to account for development is the control of enzyme specificity by genes We should still be left facing the problems presented by the strategic results of development, the appearance of differentiated tissues and organs And it is by no means clear that, in this connection, there would be any relevance in the question of exactly how the enzyme specificity is influenced

Some fifteen or more years ago, for example, the structure and functioning of genes was usually discussed in terms of physicochemical ideas, largely due to Astbury, according to which both

their main constituents proteins and nucleic acids had a fundamentally linear arrangement, with closely similar spacings of the units along the thread Recently Watson and Crick have suggested that the nucleic acid of the genes is more probably in the form of two intertwining spirals This model does not provide quite so simple a picture of the gene, since we are confronted with the difficulty of seeing how two intertwisted threads can not only reproduce themselves but then succeed in separating from one another But even when this difficulty is overcome—and it does not seem insuperable—the change in our view of the basic structure of the gene seems to make no difference whatever to our understanding of the strategic results of gene-action which were mentioned above, it appears, in our present state of ignorance, quite irrelevant to them

Again if we decide that selection operating on the genetic system of a population can bring about gradual alterations of the metric characteristics of its individuals it is more or less irrelevant to the evolutionary consequences of these changes whether they depend on a special class of gene-loci, such as the heterochromatic polygenes invoked by Mather, or on a special type of allele, such as the iso-alleles of Stern and others, or whether there is nothing very special about the genes at all, except that their effects are rather small Again the tactical problems, of immense interest as they are, and fundamentally important in their own context are largely irrelevant to the strategic questions The essential point at the present stage of evolutionary theory, is the adequacy of our two basic concepts—natural selection and the randomness of mutation It is in the main with the strategic aspects of development and evolution that the remaining essays in this book are concerned

CHAPTER 2

THE CYBERNETICS OF DEVELOPMENT

EMBRYONIC DEVELOPMENT is a highly complex process For purposes of discussion one may distinguish three major types of phenomenon which enter into it The first is the process by which the various parts of the germ, which originally seem alike, come to exhibit clearly apparent differences, one part developing say into the eye, another into the brain, a third into the intestine, a fourth into notochord and so on This process may be called 'regionalisation' It is also sometimes referred to as 'segregation', but that term tends to carry implications as to the method by which the process occurs since it suggests that the underlying mechanism is a sorting out of elementary units which were originally mingled haphazardly together In German the word 'Sonderung' is often used 'Areal bildung' is also used, and Lehmann has suggested that the word 'arealisation' might pass into international usage for the process This word is however, much more clumsy in English and little more at home in German, than 'regionalisation', which therefore seems preferable Other expressions which have been suggested, such as 'ooplasmic segregation' or 'plasmatic segregation' apply rather to the special case of regionalisation within the uncleaved egg and lack adequate generality

A second major developmental process is that by which any one particular region gradually changes its character A part which is developing into the notochord in an amphibian egg passes through the stages of roughly spherical cells, of more cuboidal cells firmly attached to one another, of very flattened cells lying side by side like a pile of coins, and finally the cells become more and more swollen by the appearance of an intra-cellular fluid Such processes of change within the derivatives of a given piece of the embryonic material may be referred to as 'histogenesis'

This name which refers to the production of particular tissues, is only fully appropriate when the process occurs in a multicellular embryo when it will characterise some group of cell lineages Similar progressive changes may affect a particular region within a single cell For instance, the gradual change in the character of the nodes of the macronucleus of Stentor described by Weisz would seem to belong to this category of processes And again the loss of regulative power in the early stages of development of an egg such as that of *Cerebratulus* (Horstadius) may well involve not only increasing regionalisation (i e increasing differences between the various regions) but also a process of change occurring within each region Strictly speaking, to cover such cases one should use a word which refers in the most general possible way to the development of the innate character of the piece of material in question, possibly 'physiogenesis' would be etymologically adequate, but for common use the better known 'histogenesis' will serve well enough

The word 'differentiation' is commonly used ambiguously to cover both these two categories we have just distinguished. Sometimes it refers to the arising of differences between the spatial parts of an originally homogeneous whole, at other times to the temporal change of character of one and the same spatial part One can continue to employ it in contexts where it is not important to distinguish between these two, but there are many occasions when it is liable to lead to misunderstanding of the two uses of it, the second, in which it means histogenesis, seems the more appropriate

The third major component of development is the moulding of a region of the egg into a definite shape, such as that of a limb or an eye or some other organ This is essentially a physical process and must involve the action of forces, arising from the chemical processes of differentiation, which change the spatial configuration of the material It is normally spoken of as 'morphogenesis'

In any concrete instance of the development of a particular part of an egg all three processes, of regionalisation, histogenesis and morphogenesis are likely to occur together For instance, in the development of a vertebrate limb bud there is a regionalisation,

by which a number of condensed regions of mesenchyme appear as the rudiments of the cartilages within the originally homogeneous mass of tissue, there is histogenesis, by which these regions gradually develop the characteristics of cartilage and later of bone, and the rest of the mesenchyme that of the muscles, etc , and there is morphogenesis, both in the original regionalisation of the cartilage rudiments in a definite pattern and then in the acquirement of specific shapes by the bones and muscles Such a complete process of development involving all three major processes may be referred to as 'individuation', since it confers on the developing material the particular and specific individual character of the organ into which it is developing

The major empirical fact about the development of animals—a fact which has no theoretical inevitability, but which is so obtrusive that only the crudest observation is necessary to establish it—is that the end-products which it brings into existence usually vary discontinuously The tissues of an animal are in most cases quite sharply distinct from one another, skin, nerve, muscle, lung, kidney, etc , with of course many sub-types in the bodies of highly evolved and complex creatures, but with very few kinds of cells which could be considered as providing a range of intermediates connecting two of the major varieties Similarly each organ has its well defined and characteristic morphology, and when, in an aberrant individual, it varies from the norm, it tends as Bateson (1894) emphasised many years ago, to appear in some other almost equally definite shape As Bateson wrote (p 42) 'To employ the metaphor which Galton used so well (in his *Natural Inheritance*)—and which may prove hereafter to be more than a metaphor—we are concerned with the question of the positions of Organic Stability'

Since the time at which Bateson was writing, the fact of discontinuity in the organic world has been generally accepted as the basis from which we have to build up the science which deals with variation between individuals—that is the science of genetics *It is, perhaps, not so widely recognised that* discontinuity has also become the focal concept in the causal study of embryological development In epigenetics, as this branch of biology is

sometimes called, most recent theory and experimentation has centred round the notion of 'determination', an idea which in effect implies that at some early stage in development certain major discontinuities between tissues or organs become established The question of how this comes about is, for epigenetics, no less crucial than the problem of the origin of species for evolution and equally, no more the whole story

It is perhaps advisable to emphasise at the outset of our discussion that the most characteristic feature of development is the occurrence of continuous and more or less gradual change This may seem too obvious to require mention, but we shall have to return to the point when considering some of the technical terms which have been proposed (for instance, 'homeostasis', cf p 41). It might be suggested also, that this fact is sometimes lost sight of in the discussions, which will not be taken up in detail in this book, on the relevance for development of the mechanisms of 'cell heredity' which operate in micro-organisms Embryonic cells appear to be inevitably undergoing processes of alteration, and the factors which control differentiation do so by steering the changing systems into particular channels By contrast, the majority of studies on heredity in micro-organisms deal with the transmission from one cell to another of stable end-states (e g the presence or absence of an enzyme or antigen) The passage of such a character from a cell possessing it to one in which it previously did not occur actually involves two phases a first phase of transmission, by some mechanism such as transformation, transduction or the like, and a second phase in which the transmitted determinant causes an alteration in the recipient cell It is the second of these which provides the closer parallel to the phenomena of development, but it is in relation to the first that the major recent advances have been made The actual processes of cell-alteration in micro-organisms, as we see them in induced enzyme synthesis or the control of the *Paramecium* antigens, for example, remain hardly less obscure than embryonic development itself

The fundamental mechanism must be one by which the different cytoplasms, or ooplasms, which characterise the various

regions of the egg, act differentially on the nuclei so as to encourage the activity of certain genes in one region, of other genes in other places (Fig 1) Such specific activation of particular genes at

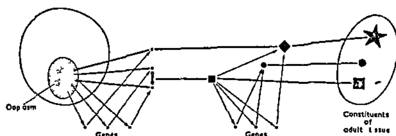


FIGURE 1

react with other genes) Certain cytoplasmic substances are produced, some of which react together, and at a later stage in development these again react with genes (the same or different ones), with the production of new substances and so on until the final constituents of the adult tissue are formed In the diagram, one of the substances (indicated by a square) acts as a 'histogenetic key substance' (cf p 55) since its interaction with the genes is responsible for eliciting the formation of the substance indicated by a circle and for the transformation of that shown finally as a star

certain times and places can actually be observed visually in favourable cases, for instance in the important work of Pavan (1954), Mechelke (1953) and Beerman (1952) on the polytene chromosomes in various tissues of chironomids The fact of differential activation of genes is, then, scarcely in doubt But there has been as yet little discussion of how we may envisage such a process in chemical terms There are innumerable different types of kinetic system which might be supposed to be in operation I have recently (1948, 1953, 1956) given a short discussion of one of these, and some further exploration of it may provide a rather more definite picture of the general character of the system with which we are confronted What we need to understand, in the

first place, is how originally slight differences in the materials available to the genes in different parts of the egg can become exaggerated and produce large and clear-cut differences between the final tissues The system of ideas to which we shall be led is in many ways very reminiscent of the notion of Organic Stability which was in the minds of Galton and Bateson some sixty years ago

The exaggeration of initial differences

Let us consider, at first, two substances P and Q, which are being formed out of the raw materials A, B and C, for the supplies of which they compete To give the simplest possible picture of such a competition, suppose that P is formed from A and B, while Q is formed from B and C Again for the sake of simplicity, let the reaction constants be the same for the two syntheses, as shown in Fig 2 and let us suppose that A, B and C diffuse into the system at rates proportional to the difference in their concentration inside (A, B C) and outside (a, b, c), while P and Q are removed at rates k_3 Then for the rates of change of the various components of the system we shall have a set of equations

$$\frac{dA}{dt} = k(a-A)-k_1AB+k_2P$$

$$\frac{dB}{dt} = k(b-B)-k_1AB+k_2P-k_1BC+k_2Q$$

$$\frac{dC}{dt} = k(c-C)-k_1BC+k_2Q$$

$$\frac{dP}{dt} = k_1AB-k_2P-k_3P$$

$$\frac{dQ}{dt} = k_1BC-k_2Q-k_3Q$$

The system will change progressively The course of the change is complicated to describe in detail, but we can discover something about the general characteristics of the system if we consider only the final steady state, when no further change is occurring, and

the right-hand side of each equation is equal to o Under these conditions it is easy to show that the ratio P/Q will be equal to a/c That is to say, if in one region of the egg, the available supplies of A are increased in comparison with those in some other region, then the steady-state concentration of P in the first region will be increased in exact proportion That is, of course, not very surprising And it hardly seems to provide much enlightenment as to the

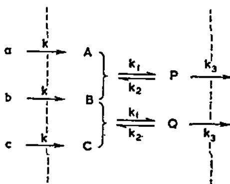


FIGURE 2
Two competitive synthetic processes in an open system

mechanisms of differentiation What we seem to meet in embryology are situations in which small initial differences lead to large divergences in later development To account for this, we need something more complicated than the very simple system we have just discussed.

As a first step towards a more adequate picture, let us suppose that the coupling of A and B to form P, and of B and C to form Q, are autocatalytic processes, i e are speeded up by the presence of already-formed P and Q This is a simple form of a 'feed-back' mechanism Our equations will now be

$$\frac{dA}{dt} = k(a-A)-k_1PAB+k_2P^2$$

$$\frac{dB}{dt} = k(b-B)-k_1PAB+k_2P^2-k_1QBC+k_2Q^2$$

$$\frac{dC}{dt} = k(c-C) - k_1QBC + k_2Q^2$$

$$\frac{dP}{dt} = k_1PAB - k_2P^2 - k_3P$$

$$\frac{dQ}{dt} = k_1QBC - k_2Q^2 - k_3Q$$

At the steady state, we find a relation between P and Q of the form

$$(kk_2c + k_3^2)P = (kk_2a + k_3^2)Q + kk_3(a-c) \qquad (1)$$

(Note that although the dimensions in this look a bit odd at first sight, k and k_3 are simple diffusion constants, while k_1 and k_2 are rate constants of third- and second-order reactions)

Now if k_3 is small compared with k (i e diffusion out of the system is slower than diffusion in), then we can neglect its higher powers, and we find

$$P = \frac{a}{c}Q + \frac{k_3}{k_2}\,\frac{a-c}{c} \qquad (2)$$

Thus if initially in a certain region the supply of A is increased relative to that of C, we find that P will be increased relative to Q by something more than a proportionate amount, the excess being expressed by the last term on the right And if the rate of removal of P (that is k_3), is greater than the rate at which P breaks down again into A and B, (that is k_2) this excess can be considerable We can also see from expression (2) that the exaggeration will be the more important the smaller the absolute values of P and Q, and these will also be reduced if k_3 is fairly large, so that P and Q are rapidly removed

Without going into further details, we can see that if two autocatalytic processes compete for raw materials, we may under some conditions find that an initial change in the supply of the materials produces an exaggerated effect on the steady-state concentrations of the synthesised products, and thus on the rates at which these products can be made available outside the system

If we suppose that a, b and c are the raw materials out of which

two genes manufacture their immediate products P and Q, we have now developed a picture by means of which we can see how change in the concentrations of these raw materials leads to exaggerated differences in the rate at which P and Q are passed out of the nucleus into the cytoplasm I have previously suggested this model, without going into such detail concerning it (Waddington 1948)

Canalised paths to alternative end-states

When we consider histogenesis as well as regionalisation we find there are certain other major requirements which the differentiating system has to fulfil We have to account not only for the exaggeration of initial differences but for two further points Firstly, the end products which are produced (the adult tissues) do not shade into one another through a whole range of intermediates but are sharply distinct—liver tissue, kidney tissue, muscle tissue, nervous tissue and so on—with only a rare and sporadic occurrence of anything which can be considered as an intergrade Secondly, the whole course of development from the initial stage in the egg up to the final adult condition is a 'most favoured path', that is to say if a mass of material is developing along one such path and is at some time during the course of development forced out of it by some experimental means, it will exhibit 'regulative behaviour' and tend to return to the normal path To express this character I have spoken of such paths as being 'canalised' or 'buffered' (Waddington 1941, 1942) The phenomenon is perhaps most easily exhibited in relation to morphogenesis, in which it is well known that an organ rudiment which is cut in half, or from which a piece is removed, may nevertheless often succeed in forming a well defined organ There is, however, little doubt that essentially the same phenomenon is exhibited by the chemical processes of histogenesis Consider, for instance, the effect of lithium or similar acting substances on the mesoderm of the amphibian egg The overall effect is that part of the presumptive notochord may be converted into somite mesoderm This must indicate that the mesoderm has been affected, and the initial effect can scarcely be supposed to be confined to the part which is

eventually converted, but must apply to the mesoderm as a whole Since some of the presumptive notochord nevertheless succeeds in developing into normal notochordal tissue, we must conclude that it has been able to compensate for the initial abnormality produced by the lithium and return to the normal canalised path of notochordal histogenesis Again, dominance of a genetic factor over its recessive allelomorph can be regarded as indicating that the developmental processes affected by it are able to compensate for the initial abnormality caused by having one recessive allele and to get into the canalised path which would lead to the normal dominant end result It is, of course, well known that dominance occurs for genes affecting chemical processes of differentiation as well as those which produce morphogenetic effects

Another essential point about histogenesis (and morphogenesis) is that the *degree* of canalisation is under genetic control That is to say, individuals of some genotypes show a more powerful tendency to regulate to the normal canalised paths of development than do others This can be clearly shown if one applies an environmental stimulus to a group of developing individuals, some will be rendered more or less abnormal, to various extents, while others may be able to adjust themselves fully to the situation and will appear completely unaffected Again, it is well known that the degree of dominance can be controlled by the remainder of the genotype

In very general terms it is clear that the occurrence of sharply distinct differences between the final end products of development must involve some form of threshold processes This term is frequently used in too vague a sense to give one much confidence that one understands the nature of the underlying systems of reaction We can look rather deeper into the matter, and pose perhaps more cogent questions, if we carry further the consideration of systems of differential equations such as those discussed above In fact the model of competing autocatalytic processes, which was mentioned there, clearly already provides, in the exaggerated response to slight changes in the initial concentrations, much of what is required to account for the appearance of distinctly different end ,

It is, however, by no means the only model which might be appropriate As Delbruck (1949) has suggested, there might be direct interactions between the two synthetic processes These are perhaps most simply formulated by suggesting that P is destroyed at some rate proportional to the concentration of Q (and vice versa) The equations for dP/dt and dQ/dt will then contain terms in PQ If we regard the system as closed, rather than open as was the system discussed above, and if the supplies of raw materials are taken as constant, the equations which result are of the same type as those which arise in the study of the growth of two populations of animals which compete with one another for a limited food supply Lotka (1934) had discussed the relatively simple situation of two populations (or substances) for which the equations take the form

$$\frac{dP}{dt} = m_p P - k_p P^2 - k_{pq} PQ$$

$$\frac{dQ}{dt} = m_q Q - k_q Q^2 - k_{qp} PQ$$

He shows that according as $m_p k_q$ is greater or less than $m_q k_{pq}$, and $m_p k_{qp}$ greater or less than $m_q k_p$ so the final state of the system is either wholly P, or wholly Q, or a certain fixed ratio between them, or finally the system is one which will finish up either entirely P or entirely Q according to the initial concentrations of these substances

Again Kostitzin (1937) discusses a somewhat similar set of equations which he takes to represent two species competing for a food supply which consists of another species which multiplies in the normal way, but which could equally well represent two autocatalytic substances which interfere with one another and also compete for a raw material which is supplied at a more or less exponential rate In this case also he shows that, under some conditions, the system will be such that the initial conditions will determine whether it goes wholly in the direction of one of the competing elements or in that of the other Denbigh, Hicks and Page (1948) have also discussed the existence of alternative steady states in simple open systems of autocatalytic reactions

In the most general case, when there are many interacting processes (e g the processes started by each gene in the genotype) we shall have to consider systems of simultaneous differential equations of the kind

$$\frac{dx_i}{dt} = f(x_{i\ j}) \qquad i,j = 1 \rightarrow n$$

Any such system of n equation will lead to p^n final or steady states where p is the order of the functions $f(x_{i\ j})$ above These steady states are defined by the solutions of the equations

$$f(x_{i\ j}) = 0$$

obtained by setting the rates of change of the variables equal to zero Some of these steady states may be unreal, in the sense that they involve negative quantities of the variables Others may be unstable It is only those which are stable, and for which all the variables are positive (or zero), that represent possible end states of canalised processes of development

Unfortunately the mathematical apparatus does not yet seem to have been developed which would enable one to discuss in any detail the conditions necessary to give rise to such states Some years ago Kostitzin (1937), who was interested in the application of such equations to the population dynamics of competing or interacting animal species, discussed shortly the case in which all the functions were of the second order He showed that such a system may be expected to exhibit 2^n alternative steady states some at least of which are likely to be stable, and he pointed out that in the situation in which there exist several compatible steady states the final one which is actually achieved will depend on the initial values 'in the sense that each steady state is surrounded by a region under its domination and if the initial state is found in one of *these regions one* will reach eventually the corresponding steady state In other cases on the contrary, the values of the final state may depend directly on those of the initial state ' It is clear that the first case corresponds rather well to what we have expressed as canalisation towards distinct alternative end conditions

since it implies that the final normal end product will be achieved in spite of minor variations in the conditions at the initial or during the early stages of development

I have not been able to discover any mathematical investigation which gives one any more insight into the conditions which lead to the appearance of such compatible steady states, each controlled by a region of initial conditions It has been pointed out (Denbigh, Hicks and Page 1948) that if we have any reaction sequence consisting of many links which are in dynamic equilibrium (e g



etc) a slight change in the concentration of one component, such as *C*, will tend to become distributed amongst all the elements in the sequence, the partitioning of the disturbance among them depending on the reaction constants This phenomenon in itself will provide some degree of 'buffering', since a considerable alteration in some earlier step in the sequence will have a lesser effect on the terminal stage But it hardly seems that this can be sufficient to account for developmental regulation, which often seems completely to annul the disturbance caused by an early interference

On general grounds it seems probable to me that such canalisations are more likely to appear when there are many cross links between the various processes, that is to say when the rate of change of any one variable is affected by the concentrations of many of the other variables Such cross-linkages between variables must certainly be invoked to explain the control of the canalisation of the development of one character by many genes

Equations of this kind have also been recently discussed by Ross Ashby (1952) The greater part of his discussion is limited to systems of linear equations, i e those of the first order He makes the interesting point that the greater the number of variables involved, the less is the probability that any steady state will be stable This would seem to make it difficult to explain the occurrence of canalised end states in which the degree of canalisation is affected by large numbers of genes Ashby, however, does not seem to have taken account of the fact that an increased number of

variables also leads to an increase in the number of alternative steady states produced by the system It seems rather likely that the decrease in the probability that a steady state will be stable is compensated for by this increase in the number of states, so that the number of stable steady states may not be very greatly affected by increase in the number of variables concerned

The discussions of these matters by Kostitzin and Ashby have been given in terms of closed systems, that is to say systems which are affected neither by an influx of material from outside nor by an efflux out of the system Living animals, however, must really be open systems, since they both take up raw materials from their surroundings and give out waste products (Bertalanffy 1949) The equations necessary to describe an open system are not basically different from those that apply to closed systems, but the stationary states will refer not to static conditions but to situations in which there is a flow of material through the system which leaves the concentration of the various components unchanged

The flow-sheet of an open system—or of a closed one for that matter—does not contain any term which implies the possibility that one of the reactants may totally disappear from the system It would be wrong, however, to deduce from this that a hypothesis which suggests that gene action can be considered in terms of open systems cannot contemplate the eventual disappearance (or complete inactivation) of certain genes from particular tissues Genes, in fact, can be introduced into our open system picture in two different ways If, to take the first of these, one concentrates attention on the gene-activities which steer the developmental processes, then the genes can be considered as catalysts which determine the rates at which the various reactions will occur The flow into and out of the system does not then pass through them, they merely modify its speed There is nothing to prevent us attributing to them rules which govern their stability It might be, for instance, that each gene persists only for a definite lifetime, to be measured presumably in cell-divisions A more probable suggestion is that the integrity of a gene is safeguarded by the availability of suitable substrates, in the absence of which it tends

to undergo a degenerative change of the nature of a denaturation

A second aspect of the activity of genes is their self-reduplication In manufacturing a replica of itself, a gene obviously requires raw materials and gives rise to a product, it is then one of the items on the flow-sheet through which the flow passes In so far as this is true, it cannot vanish wholly from the system But it must be remembered that the actual development of a multicellular organism is not as continuous a process as is implied by the comparatively crude picture we have been discussing The continuity is broken by intermittent steps of cell division The possibility arises that, in some tissues a gene may proceed with its reduplication-process at a slower rate than its fellows, and fail to complete it by the time the next division occurs It might then be absent from one of the daughter cells and all its progeny

There must, one imagines, be some rather close relationship between the activity and the reduplication of a gene, but we still know very little about it Is the primary gene-product chemically very similar to the gene-replica? If so, both replication and activity will demand the same or similar raw materials, the same substrates are likely to safeguard the gene in both its aspects, and an absence of the appropriate materials for the gene's activity may lead to the loss of its capacity to reproduce itself On the other hand, if there is more difference between the processes of replication and production of primary product, the former may persist throughout a lineage of cells in which the latter does not occur Finally, there may be not a synergism but an antagonism between the two aspects of the gene's activities This is, perhaps, suggested by the [illegible]

namely cell-division

There is not yet sufficient evidence for one to decide whether all genes do persist, in fully functional form, in all cells of the organism In the first place, it is relevant to notice that there are several grounds for thinking that genes are active, in the sense of producing their primary products—and therefore *a fortiori* of reproducing up to that stage in the life history—in many more

tissues than would be guessed at first sight For instance Demerec (1934 1936) found that in a surprisingly large number of cases the complete absence of a short section of chromosome containing only one or two genes is completely lethal to the cell containing the deficiency, even when there was no other evidence that the [illegible]

shown to be actually operating in a much more widespread manner by combining them with some other mutant genes which renders the development of other organs less well canalised and more easily brought to exhibit visible abnormality

On the other hand it can by no means be taken yet as certain that epigenetic systems are in all cases entirely open ones, from which the original reactant genes cannot wholly disappear For instance, King and Briggs (1955) have transplanted nuclei from various tissues of the frog embryo into uncleaved and parthenogenetically activated eggs from which the female pronucleus had been removed, they found that the nuclei of many early tissues could make possible the development of a complete embryo, and must therefore have contained a full set of genes capable of activity, but some of the endoderm nuclei were incapable of supporting the differentiation of other tissues, although able to continue reproducing themselves and dividing These restrictions in the potentialities of the nuclei may perhaps find their explanation in the complete disappearance, or at any rate total inactivation, of those genes which are not required in the differentiated cells from which the nuclei were taken, although there are of course other ways in which the phenomena could be accounted for

Developmental pathways

In the study of development we are interested not only in the final state to which the system arrives, but also in the course by which it gets there In order to study these developmental pathways algebraically we should have to integrate the sets of equations by which the system is described It is usually impossible to do this in any general way, although for any particular system

solutions could be computed numerically, as Turing has done in a few cases For purposes of general discussion, however, we must fall back on a mode of expression which may be called geometrical rather than algebraic

A system containing many components can be represented by a point in multidimensional space, the co-ordinates of the point in each dimension representing the measure of a particular component A space of this kind is known as a phase space As the composition of the system changes the point will move along a certain trajectory In a canalised system of the kind we have been considering, trajectories starting from any point within a certain volume will converge to a single end point which is the corresponding steady state, while trajectories starting within some other volume will converge on a different point (Fig 3)

A multidimensional phase space is not very easy for the simple-minded biologist to imagine or to think about Thus several people have tried to formulate less precise but more vivid diagrammatic representation Ross Ashby (1952) has given a discussion in terms of two dimensions He envisages the state of the system as represented by a point on a plane, and the dynamic conditions corresponding to the sets of differential equations as determining how the point will move He was interested in finding a system whose composition would eventually reach a final state in the neighbourhood of some predetermined value If the original system did not give an end state in this neighbourhood he supposed it to be changed into a new system by some threshold-like alteration in the value of one of the components This process of sudden alteration by a change in what he speaks of as a 'step function' would continue until the system fulfilled the desired condition of converging on to some point in the assigned neighbourhood

This picture does not seem very appropriate for a consideration of development In that context one particular system is given initially by the genes in the nucleus and the regionally differentiated cytoplasm, and we are concerned with the whole set of end states characterising it The true representation of this, as has been stated, is a multidimensional space, subdivided into a number of

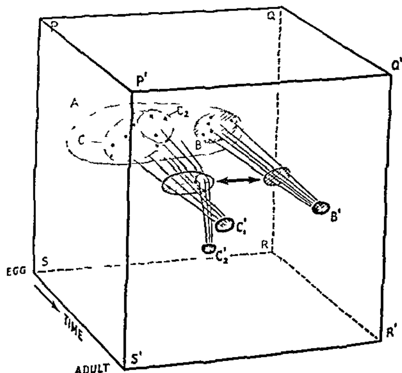


FIGURE 3

A phase-space diagram of development The time axis runs perpendicular to the paper, from the plane *PQRS* at the time of fertilisation to *P Q'R'S'* in the adult The other two dimensions represent the composition of the system The composition of the various parts of the egg (which in this case varies continuously, in a 'gradient' manner) originally lie within the area *A* One region of the egg, with composition *B*, develops along a series of trajectories which converge towards the adult tissue *B'* Another region, with composition C_1+C_2 also begins to develop along a converging set of trajectories At some stage during development, physical contact (heavy double arrow) occurs between the *B* region and C_2, a reaction occurs by which the C_2 trajectories are diverted so as to converge on C_2, this represents an induction.

regions, such that trajectories starting anywhere within one region converge to one certain end point, while those starting in other regions converge elsewhere (Fig 3) I have tried to give a simple model in three dimensions which will correspond with this to

some extent. Consider a more or less flat, or rather undulating, surface, which is tilted so that points representing later states are lower than those representing earlier ones (Fig. 4). Then if some-

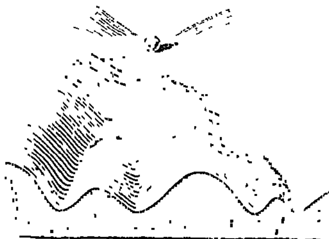


FIGURE 4

[illegible]

a second alternative is offered; along the path to the left, the main channel continues leftwards, but there is an alternative path which, however, can only be reached over a threshold.

thing, such as a ball, were placed on the surface it would run down towards some final end state at the bottom edge. There are, of course, not enough dimensions available along the bottom edge to specify all the [illegible]

so on for each type of tissue or organ. Similarly, along the top edge we can suppose that the points represent different cytoplasmic states in the various parts of the egg. Or we could represent the various different initial conditions by imagining various degrees of bias on the balls which are to run across the surface (cf. Waddington 1954*b*, Fig. 2).

The essential character of regionalisation is that trajectories starting from the various points along the top edge find themselves converging on one or other of the organs marked along the bottom The canalisation which we have described as characteristic of histogenesis implies, firstly, that the convergence takes place to end states which are sharply distinct from one another, and secondly, that if while the system is moving along a certain trajectory it is pushed slightly out of its course it will tend to compensate for this disturbance and to reach eventually the same end state as it would normally have done These two characteristics have been expressed visually by the modelling, which together with the initial tilt, controls the course of trajectories We have to picture the surface as grooved by valleys, each leading to one of the normal end states The number of separate valleys must increase as we pass down from the initial towards the final condition I have earlier (1940) suggested the name of 'the epigenetic landscape' for this diagrammatic representation of the developing system

The epigenetic landscape

Although the epigenetic landscape only provides a rough and ready picture of the developing embryo, and cannot be interpreted rigorously, it has certain merits for those who, like myself, find it comforting to have some mental picture, however vague for what they are trying to think about For instance, it makes one reflect that there may be regions at upper levels which are almost flat plateaus from which two or three different valleys lead off downwards These, in fact, correspond to what we know as states of competence, in which embryonic tissues are in a condition in which they can be easily brought to develop in one or other of a number of alternative directions Again, the model immediately suggests that one ought to consider the degree of canalisation of any particular path of development Has the valley a flat bottom and gently sloping sides? If so, there will be only rather a slight tendency for a developmental trajectory, when displaced from the valley centre, to find its way back there again, the final adult character will be easily caused to vary by minor fluctuations in the

conditions under which development occurs On the other hand, if the valley bottom is very narrow and the sides steep, it will be more difficult to push the trajectory away from its normal course and it will quickly return there, unless indeed it has been pushed over the brow of a watershed either into another valley or on to a plateau which represents some aberrant conditions intermediate between one organ and another We shall later make use of this model of the epigenetic landscape to discuss questions of this kind in a context in which it is more important to employ a system of thought which is flexible and of wide application than to search for a precise formulation of a narrower viewpoint

There are three major types of developmental system, depending respectively on segregated ooplasms, on gradient systems, and on inductive relationships They will be represented by epigenetic landscapes of rather different kinds In an egg in which definite ooplasms are clearly separated from one another before fertilisation occurs, or shortly after, the landscape will have the form of distinct valleys even at the time at which development begins On the other hand, in an egg with a gradient system, such as that of the echinoderms, the uppermost part of the landscape will be a single broad valley, which only gradually divides up into separate channels Eggs in which induction is important, such as those of amphibia and other vertebrates, will have a landscape which may [illegible]

their characteristic feature is, however, that at later stages the processes going on in one channel influence happenings in some other channel, causing a division of the latter into an induced and an unaffected portion. The effect of such an inductive influence of the tissue in one pathway on that in another can be regarded as mathematically equivalent to some alteration in the initial conditions of the processes in the induced area

It is worth emphasising again that the epigenetic landscape is a representation in three dimensions of properties of the system which really involve many dimensions of phase-space However it does successfully incorporate some features of this system which

have not yet been mentioned We spoke earlier of trajectories in the phase space as converging on one or another definite end-point As a matter of fact, if a process of embryonic development is disturbed, it usually returns to normality some time before reaching the adult condition Its trajectory, that is to say, converges not merely to the normal end state, but to some earlier point on the path leading towards the steady state This is well symbolised by the epigenetic landscape If a ball, running down one of the valleys, were pushed partway up the hillside, it might well reach the valley bottom again before the slope of the valley flattens out as it reaches the adult steady state Such a system exhibits a tendency towards a certain kind of equilibrium, which is restored after disturbance, but this equilibrium is not centred on a static state but rather on a direction or pathway of change We might speak of such an equilibrium-property as a condition of 'homeorhesis', (ρηω, to flow) on the analogy with the well-known expression homeostasis, which is appropriate when it is an unchanging state which is maintained

Oddly enough, I can discover no technical word meaning a pathway of change which is equilibrated in the sense that the system tends to return to it after disturbance One would have expected that such a notion was a commonplace of the kinetics or cybernetics of polyphasic systems I should suggest that a reasonably short and suitable word would be 'creode', from the two Greek roots χρη, it is necessary, and ὁδος, a route or path A creode, then, is a representation (e g by a trajectory in phase space) of a temporal succession of states of a system characterised by the property that the system, if constrained to move slightly away from the creode, will tend to return to it The path followed by a homing missile, which finds its way to a stationary target, is a creode

A creode has two main characteristics which require notice The first, which one might call the creodic profile, corresponds in the epigenetic landscape to the slope along the valley bottom, as one proceeds from the initial to the final state of the system This is a concept which is not easy to make quantitatively precise in the three dimensional terms of the epigenetic landscape, since in that

diagrammatic representation of the system it is more or less arbitrary what scale is used to measure the progress of development. In vague enough terms, one can say that in some periods [illegible] flatter ones I do not intend to attempt here the task of making such notions more precise, since nothing I wish to say later involves them

That is not to say that there are no interesting questions concerning creodic profiles which almost plead for consideration I will mention, very briefly, only two of them In the first place, systems of simultaneous differential equations such as those on p 22 often have solutions which approach the steady state through a series of damped periodic fluctuations It would at first sight seem most improbable that processes of this kind occur in developmental systems They would involve a series of alternating phases of the synthesis of an over-large quantity of some substance followed by its breakdown to a lower concentration than the final one If such processes really do not occur during development, this fact would limit the kinds of equation which can form part of the system However, Kavanau (1954), studying the protein synthesis in the sea-urchin egg, describes a system which is certainly very like a relaxed oscillation. Another problem is the nature of what we have referred to as final steady states The phenomena of 'recapitulation', in the broad sense, show that genetic changes do not always alter the course of a creode, they may instead cause the steady-state condition to arise at an earlier or later stage in it How does this happen? It may be that the solution will be found to involve the fact that to speak of the adult condition as a steady state is to some extent an oversimplification, since developmental change continues at a slow rate throughout adult life, leading eventually to senescence But to follow these problems would lead us too far from our main theme

The other important property of a creode is that which corresponds to the shape of the cross-section of the valley in the epigenetic landscape This is a most important characteristic It

indicates the strength of the tendency of the system to return to normality, that is, the intensity of its homeorhesis We will have much to say about it later. It may be referred to as the 'homeorhetic cross-section' of the character in question, or as the 'canalisation cross-section' Often we shall speak of the intensity, or degree, or steepness, of the canalisation of an adult feature, and all such phrases refer to the homeorhetic cross-section

In discussing homeorhetic cross-sections, we may find ourselves confronted with a difficulty similar to that noticed above in connection with the creodic profile, namely how to determine the scale of measurement to be used for the characters involved It is easy to compare the canalisations of the same feature of two different animals or races, since, whatever unit of measurement is chosen, it can be used in both cases But one often feels tempted to institute comparisons between organs, asking for instance, whether the number of scutellar bristles in *Drosophila* is more narrowly canalised than the body size But how can we compare a certain amount of variation in bristle number with a variability of size? The same difficulty arises if one merely enquires whether one character is more variable than another, not considering whether the variability is due to the availability of more diverse alleles or to lesser canalisation A meaning can only be attached to such phrases in a particular context, in relation to which an appropriate measure of variability can be defined If, for instance we are concerned with the utilisation of variation for the evolutionary divergence of species, then the characters can be measured in units derived from the extent of the phenotypic differences between members of the species group, or in some similar way which seems appropriate to the particular question at issue Comparisons between the variabilities or canalisations of different characters may therefore be meaningful and interesting in definite contexts, even though they cannot be given any absolute sense (See p 138)

It is important to realise that the comparatively simple orderliness of the epigenetic landscape—its restricted number of valleys with their branching-points and characteristic contours—is a property of a higher order dependent on an underlying *network*

of interactions which is vastly more complicated The cells proceeding along any developmental pathway must have a metabolism of some corresponding complexity The mere histological appearances of developing cells is sufficient to demonstrate this, and recently new techniques, such as chromatography and the use of tracers and metabolic antagonists, have begun to provide a more detailed picture of the situation But genetics still gives us more insight into the real complexity of apparently simple epigenetic processes than does biochemistry In well-studied forms such as *Drosophila*, we know that the development of any given organ such as the wing is influenced by very many genes, which operate in sequence and in many cases interact with one another (cf Waddington 1940) Since each gene must be regarded as a distinct chemical entity, the path of development as it is observed by the anatomist must be viewed as the resultant of all the very numerous processes in which these genes are involved in the cells concerned

Fig 5 is an attempt to express this consideration in a visual form, it is not, of course, intended to be interpreted in any literal way, but it may serve to bring home the point more vividly to those who tend to think in images We are it might be said, looking at the under side of the epigenetic surface which is represented as a great sheet of canvas suspended at a considerable height above our heads and sloping down towards the ground in the distance This sloping surface is grooved into valleys along lines where it is dragged down by a complicated network of guy ropes, which are attached to pegs in the ground These pegs represent the genes, and the tensions on the guy ropes the chemical forces which the genes exert As the diagram indicates, the course and slope of any particular valley is affected by the chemical tendencies of many genes, and if any gene mutates altering the tension in a certain set of guy ropes, the result will not depend on that gene alone, but on its interactions with all the other guys

A final point that requires emphasis is this It is rather astonishing to find that by selective breeding from suitable individuals taken from any large population of animals one can usually be successful in controlling in great detail any chosen single path of

development. For instance, if into a population of *Drosophila* one introduces some mutant gene whose main effect is, say, on the legs, one nearly always finds that it is possible by selection to build up races in which the leg effect is either strongly or weakly exhibited,

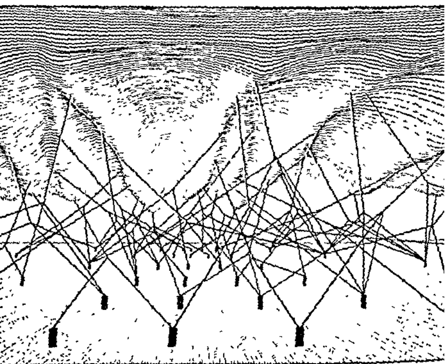


FIGURE 5

The complex system of interactions underlying the epigenetic landscape
The pegs in the ground represent genes; the strings leading from them the chemical tendencies which the genes produce. The modelling of the epigenetic landscape, which slopes down from above one's head towards the distance, is controlled by the pull of these numerous guy-ropes which are ultimately anchored to the genes.

the races not otherwise differing to any noticeable extent. Again, if one applies some strong environmental stimulus to a mixed population of *Drosophila* it is usually possible

shocks to *Drosophila* pupae at the age of about 22 hours, successfully bred from one initial population two derived strains, one of which reacted to the stimulus by producing accessory wing venation while the other reacted by failing to develop its normal anterior crossvein (cf p 183)

Such phenomena are usually explained by postulating the existence of a number of minor modifying genes It must be recognised that an explanation in terms of genes is essentially hypothetical, since in the vast majority of cases no individual genes can be precisely identified Nevertheless it seems clear that it is ultimately in terms of genes that these developmental processes must be controlled Many people, however, for instance Goldschmidt (1951) and 'Espinasse (1956), have pointed out that we are in grave danger of having to postulate a quite unreasonably large number of genes to provide modifiers which can affect all the normal and aberrant developmental processes which might be encountered We can, indeed, hardly sustain nowadays the original conception of modifying genes which supposed that they were quite inactive except on the aberrant developmental process produced by a major mutant gene or by an intense environmental stimulus In contrast to this the system of ideas which has been disc[illegible] gre[illegible] developing system processes What we now have to add to this picture is something to account for the fact that one particular path leading, say to the formation of the wing, can be modified in detail by gene changes which leave other paths more or less undisturbed It seems possible that the simplest solution to this problem is to be found by supposing that in each line of development the details of the end result depend on interactions between certain particular genes Genes consist of nucleo-protein and must be expected to have a very high degree of specificity It is not too difficult to imagine that any particular gene may exist in a large number of forms which differ only in their minor biological (possibly immunological) specificity Such forms perhaps correspond to the iso-alleles described by Stern and Schaeffer (1943) and others All the iso-alleles would have much the same efficiency in

carrying out the main work of the gene but they might differ quite considerably in their capacity to form with some other gene a compound in which this biological specificity played an important part. It seems rather probable that such gene compounds, if they play a part in development at all, would be of major importance only between certain critical limits of concentration of the reacting substances, that is to say each gene compound would become important only in certain particular lines of development. Changes in combining-specificity of genes which left their major functions little affected might therefore provide a basis for the kind of rather strictly localised modifying action for which we have been seeking an explanation.

The specificity of buffering

It is a defect of the attempt to symbolise a multi-dimensional system in only three dimensions that the buffering of any path of development is represented as though it were the same with respect to all possible kinds of environmental or genetic stresses. This is an over-simplification. It is justified only as a first rough approximation, or in cases where we are considering the effects of only one type of stress. But for generality, we must realise that there should be a specific homeorhetic cross-section corresponding to each different kind of influence which affects the system. For instance, in *Drosophila* alterations of the wing veins can fairly easily be produced by heat shocks, but the epigenetic system which controls vein development seems to be much more strongly buffered against changes in oxygen tension, no abnormalities of venation being found in flies reared at the lowest oxygen pressures possible. Similarly, if we build up, for instance by selection, a strain of animals in which some gene substitution has *only* a very slight effect (for instance, a stock in which the dominance of some gene affecting the eyes is reduced) we would not necessarily *find that the effect of other genes influencing* the same organ had also been reduced. We still have very little information about the frequency with which the canalisation of a developmental system is specific to one particular stress as opposed to providing a general buffering against a wide variety of different stresses. It

seems probable, however, that the canalisation evolved in populations under natural conditions usually involves a rather general buffering, since such populations must have to cope with variations in many environmental conditions rather than extreme stresses of any one kind such as tend to be used in laboratory experiments

Developmental noise

It can hardly be expected that any epigenetic mechanism can operate with complete precision Quite apart from any disturbances due to the external environment of the embryo, there are likely to be slight irregularities in the interactions between the different parts of the germ, which, in a sense, provide an environment for each other This is particularly noticeable when a developmental process is carried out not by a massive tissue but by isolated cells For instance, when the melanoblasts from the neural crest distribute themselves over the flanks of an amphibian tadpole, they do so in a pattern which is more or less definite but not absolutely the same from individual to individual Again in the primitive streak of the chick, the invaginating mesoderm becomes dissociated into cells which are loosely arranged and can [illegible]

behind their neighbours and finish up in that organ From quite another type of animal, one may cite the formation of hairs by isolated cells in the hypodermis of the abdomen of *Drosophila*, Reeve and Robertson (1954) found that a large part of the variation between contiguous segments in hair number cannot be attributed either to genetic factors or to environmental circumstances which affect the whole animal, but must be brought about by intangible internal sources of variation

This kind of looseness or 'play' in the epigenetic machine might be referred to as developmental 'noise', using that word in the sense given to it in information theory There is some degree of imprecision even in developmental processes which involve large numbers of cells It is certainly noteworthy that

embryonic organs are often somewhat variable at the time of their first appearance, and only gradually become regulated towards the standard The early somites of a chick embryo, for example, are often rather variable both in size and in outline The later regulation in such cases is an aspect of the canalisation of development and the earlier variability, *in normal embryos, can scarcely* be attributed to anything but developmental noise The regulation towards the standard shape of the organ is particularly marked in some experimental situations, for instance it seems that any mass of neural tissue, which at the time of its formation has a rough resemblance to some part of the brain region of the neural tube, will eventually develop into a relatively well-individuated segment of that organ (cf Waddington 1952*b*, Nieuwkoop 1955)

It is important to distinguish the *inherent noisiness* of a developmental pathway or creode from its canalisation Developmental noise will lead to the formation, in a constant environment, of adults which vary somewhat around some mean value The extent of this variation need not give any information about the magnitude of the effect on the mean which would be produced if the environment were changed, this is a function of the degree of canalisation of the creode If canalisation is represented as a valley in an epigenetic landscape, the noisiness of the system might perhaps be symbolised by the imperfection of the sphericalness of the ball which runs down the valley In practice, specially designed *experiments would be necessary to estimate the two aspects* of the system One might, for instance, measure canalisation by rearing samples of the population under different conditions, and one might attempt to assess the noise as the lower limit of the variation as the heterogeneity of the environment is reduced

Mather (1953) and Tebb and Thoday (1954), studying the difference between the number of sternopleural bristles on the two sides of a *Drosophila*, have shown that developmental noise is, as might be expected influenced by the genotype But there is no reason to suppose that its genetic control is identical with that which is concerned with the responsiveness of the epigenetic system to environmental *alterations or to* changes in some of the genetic alleles affecting the character It is therefore probably not

justifiable to take such studies as providing information about the general 'developmental stability' or 'developmental homeostasis' of the system, as these authors do, at least if these terms are being used as equivalent to developmental canalisation It seems desirable, either to restrict the term 'developmental stability' to mean 'lack of noisiness in development', in which case one could speak of selection for similarity between two homologous organs as 'stabilising selection', or to coin another word for such selection, calling it perhaps 'selection for repeatability' (cf p 72)

Matters of terminology

There are several other concepts, which share with developmental canalisation the fact that they also deal with phenomena involving equilibria Some confusion has been caused by the use of the word 'homeostasis' for a number of different ideas Classically, this word is used (e g by Cannon 1932) to refer to the capacity of an organism to maintain its physiological functioning at some definite level Thus one speaks of a homeostasis of the pH of the blood, or of its oxygen concentration For this use the word is eminently appropriate, since it carries an implication of the maintenance of a constant value of one variable in the system

The word has also been used recently (e g by Lerner 1952) of genetic rather than physiological processes In a naturally adapted population the gene frequencies will, under the influence of natural selection, mutation etc , be in equilibrium If this is disturbed by artificial selection, inbreeding, etc , it is often found that when the constraint is relaxed there is a tendency for the system of gene frequencies to return to its original state, mainly through the operation of natural selection It is to such processes that the term 'genetic homeostasis' has been applied, and here again appropriately enough, since the frequencies are maintained at the unchanged equilibrium

It will be clear that developmental canalisation is something different from either of the two former types of homeostasis, it denotes a tendency to attain something which can perhaps be regarded as an equilibrium, but in this case the processes involved are developmental rather than matters of either physiological

functioning or genetical inheritance Although the idea that the regulatory phenomena of development can be envisaged in terms of equilibria was expounded at some length a considerable time ago (Waddington 1939, 1940, 1948b), it is only recently that other authors have devoted much attention to it, and they frequently refer to it by the term homeostasis rather than canalisation The Greek word is perhaps the prettier and the more impressive, but it does not seem very apposite in this context, since there is nothing static about development, we are not dealing with the maintenance of a steady state but with the attainment of some particular end-state in spite of temporary deviations on the way there Moreover the term cannot easily be made sufficiently flexible One wants to be able to speak of different types (smooth or cusped) of *canalisation, and of different intensities (narrow, broad, etc)* The terminology employing homeostasis does not easily allow of this

The main argument against adopting the term 'developmental homeostasis' arises, however, from the fact that the evolution of a canalisation is, in some ways, antagonistic both to genetic homeostasis and to physiological homeostasis The more narrowly canalised is the development of a character, the less will changes in gene frequency come to phenotypic expression, and the less will be the tendency to genetic homeostasis Again, a high capacity for physiological homeostasis implies that when the environment is altered development will be modified in such a way that the organs can carry out their physiological functions with normal efficiency in the new circumstances, and this may necessitate a departure from canalisation These inverse relations between developmental canalisation and the other two processes can cause statements to appear highly paradoxical if the word homeostasis is used for all three phenomena and any confusion arises as to which is under consideration

Dobzhansky (1955) has recently suggested that developmental canalisation is merely one aspect of physiological homeostasis He writes "Waddington (1942, 1953) and Mather (1953) used the word 'canalisation' instead of 'homeostasis'. An explanation may here be in order Waddington's 'canalisation' is evidently a part of the general physiological phenomenon of homeostasis as

defined by Cannon (1932) Homeostatic maintenance of the 'steady state' of the organism in the face of changing environments is possible only thanks to a remarkable plasticity of the physiological machinery For example, the ionic concentration of the blood in mammals remains constant because the kidneys are working differently when too much or too little salt is ingested The 'steady state' which is maintained is that which permits the body to remain alive and to continue its development along one of the phylogenetically established adaptive paths *Functional homeostasis*, with its marvellous reversible reactions, which are so important in the maintenacne of health and well-being, thus results in *developmental homeostasis*"

This greatly oversimplifies the situation For example, urodele larvae reared in low oxygen tension become adapted to the environment by two changes, in both of which one can see an essential opposition between homeostasis and canalisation (cf. p 157 and Fig 27) On the one hand, the external gills become larger in area and their walls thinner this is a departure from developmental canalisation in order to facilitate the homeostasis of the blood oxygen level On the other hand, the rate of respiration per unit of body weight is reduced this is a departure from physiological homeostasis, presumably forced on the animal because its developmental canalisation is too strong to permit complete constancy of the physiological functioning It seems clear that in such a case it would only lead to confusion to neglect the distinction between the two concepts of canalisation of developmental end-state and homeostasis of the level of physiological functioning

For all these reasons, I shall continue to speak, in the context of development, of canalisation or "homeorhesis', using homeostasis only of the genetical and physiological situations The main objection, which might justly be urged against the term 'canalisation' is that it is derived from an analogy or metaphor, in which three dimensions are used to express the properties of a system which really *involves a multi-dimensional phase space*, it may therefore suggest too concrete an image to be suitable as a name for the abstract quality to which it refers, but this seems a less

important failing than those involved in the alternative term homeostasis

We need therefore, three separate notions in connection with biological tendencies towards equilibria physiological homeostasis, when it is some physiological state which is being held constant, development homeorhesis or canalisation when it is the course of development which is relatively invariant, and genetic homeostasis when the constant feature of the system is its set of gene frequencies

It may be advisable to say a word about certain other related expressions which have recently been employed Warburton (1955) has discussed 'feedback in development', where feedback is the term used in cybernetics for a mechanism by which a homeostatic or canalised system operates However, although one would have expected that the author would be concerned with developmental canalisations, he actually uses the term to deal with adaptive developmental modifications by which physiological homeostasis is ensured, that is to say, with the type of controlled looseness of canalisation which leads to exogenous adaptation (see p 151) In all the examples he gives, it is not the end-result of development which is invariant, or in his terms is the goal of the feedback, on the contrary, the goal is a certain level of physiological functioning, and the course of development is modified to produce organs which under the deviant circumstances, allow physiological homeostasis to be achieved (Having in effect omitted to consider true developmental canalisaiton, he naturally seems to find some difficulty in coming to grips with the notion of 'genetic assimilation', which will be discussed in Chapter 5)

Another recent technical term in this field is 'developmental flexibility', defined by Thoday (1953) as follows 'An individual or organism may be said to possess developmental flexibility either if its genotype is such that it can develop different phenotypes in different environments, each phenotype better adapted than the others to the environment that evokes it, or if its genotype is so balanced that development is buffered against environmental variables and hence apparently the same adaptive phenotype results in a range of environmental conditions' It is surely clear

that two quite different things are included in this The second half of the definition refers to developmental canalisation in our sense, and to call it 'flexibility' would seem to be putting the cart before the horse It is, admittedly, clear that in any system which returns to a determined state after disturbance, the reactions must be able to proceed with varying intensity while the regulation is actually being carried out, and it is presumably this feature which is being referred to as flexibility Nevertheless, the same type of inconstancy would be found in any dynamic system The characteristic with which we are concerned is not that the system is as changeable as any other, but precisely that its end-result is less easily altered This criticism does not apply to the first half of the definition, which does refer to something which might not too inappropriately be called flexibility, but the trouble here is that the term hardly seems precise enough What is envisaged is not merely that development is easily altered, but that it becomes modified in adaptively valuable ways, that is to say that the organism acquires exogenous adaptations (p 151), this can scarcely be conveyed without speaking at least of 'adaptive flexibility'

Thoday introduced the notion of developmental flexibility in connection with evolutionary problems The key concept, towards an understanding of which he was working, was that of fitness The relation of developmental homeorhesis and physiological homeostasis with fitness is of course a most important one, it is discussed at some length in Chapter 4 The connection is certainly not a simple one, and for that reason it leads to confusion to attempt to introduce the idea of fitness into the definitions of the epigenetic and physiological terms, particularly when these two are themselves not sharply distinguished For instance, Dobzhansky and Wallace (1953) made some experiments in which they compared against a standard strain, the viabilities of flies in some of which certain chromosomes were heterozygous while in the others they were homozygous Their results showed that 'heterozygotes are more uniformly successful in a variety of environments than are homozygotes, this suggests that the heterozygotes are better able than the homozygotes to cope with

these different environments and to maintain their internal milieu in functional order' And a sentence or two earlier they write 'We do not know which physiological processes in *Drosophila* must be maintained constant to enable the development to proceed unimpaired' But in fact we do not know whether the superior and more uniform performance of the heterozygotes was due to a greater capacity for carrying out physiological adaptations which kept the internal milieu so constant that development proceeded along its normal course (in which case the physiological homeostasis resulted in a developmental homeorhesis), or whether the development was modified in a manner which facilitated the physiological adaptation. These two alternatives are different We have to suppose that one or other of them is in fact the case, provided that we adopt the basic assumption that the fitness of the animals is directly dependent on the constancy of something or other—either development or physiological functioning This may be plausible, but there is little direct evidence for it But each of the three concepts—fitness, homeostasis and homeorhesis—needs to be grasped separately before their interrelations can be discussed with clarity

They are again confused, indeed even more completely, in a recent paper by Lewontin (1956) He in fact equates homeostasis with fitness Thus he offers as a definition that 'a genotype is homeostatic if individuals of this genotype can so adjust their physiology and morphogenesis as to survive and leave offspring in a wide variety of environments' And in summarising his work states that 'homeostasis has been defined in this paper as the property of the genotype or collection of genotypes which allows it to respond adaptively to a wide variety of environments The measure of homeostasis *is then the average adaptive value over a range of* environments' In such an interpretation the connection between homeostasis and the constancy of something-or-other has been completely lost—or rather it survives only on the basis of the arbitrary assumption that survival and reproduction of the animal depends on the preservation of constancy either of some morphological entity or physiological function This may, of course, often be the case An animal moved to a high *altitude may preserve* the

constancy of its blood oxygen pressure, and may do so either developmentally (e g by an increase in the area of lung alveoli) or physiologically (e g by a more rapid heart beat), but equally it might well survive by abandoning any form of homeostasis, allowing its blood oxygen to fall and also reducing the rate of energy output and thus the tissue requirements for oxygen (cf. Fig 28, p 158) Indeed Lewontin's own experiments (which are most interesting ones) force him to the conclusion that 'those homozygotes which are less homeostatic than heterozygotes constituted from them, show a lower variance in total bristle number', a statement which is completely paradoxical if one associates homeostasis with constancy

The evolution of systems providing genetic homeostasis

Developmental canalisation does not operate by means of selective alterations of gene frequencies Its mechanisms, although not understood in detail, must involve competitions of enzymes for substrates, mutual enhancements and inhibitions of enzyme-activated processes and the like Canalising selection is the agent, not by which canalisation works, but by which it is brought into being and evolved Genetic homeostasis, on the other hand, does operate by means of selection, which provides the actual mechanism by which the constancy referred to, the 'homeostatic condition', is maintained But just as the mechanism of canalisation is produced by evolution, so must the system whose properties lead to the existence of a greater or lesser degree of genetic homeostasis And it is interesting to enquire in what way evolution may be supposed to have brought such systems into being

Two rather profoundly different types of answer can be envisaged The simpler type of reason is one which involves no assumptions, other than those of everyday genetics, concerning special properties of the genes It would suggest that there are two situations in which systems giving genetic homeostasis would be expected

Firstly, if the population is living in a uniform environment, there will be some optimum value for any character closely involved in the fitness of the individual, but there is no reason to

suppose that, initially, this value will be exactly and exclusively attained by one of the homozygous genotypes available One can think of the population as being suddenly transferred into the environment, so that *the new optimum value of the character has* to be produced by new mutations It would be most probable that mutation would throw up alleles which bracketed the optimum, some acting too feebly and some too strongly, the nearest approach to the optimum would be found in some intermediate heterozygote The value of the character in such a heterozygote would also be influenced by alleles at other loci, acting either as modifiers of the most important locus, or as a set of genes of relatively similar importance. Robertson (1956*a*) has shown that selection for an intermediate optimum will, in a uniform environment, eventually lead to fixation of gene frequencies, that is, the optimum will eventually be attained by some homozygous form But this process depends on either the second-order selection of [illegible]

only slowly

A situation in which the population is homozygous or nearly so can perhaps be said to exhibit some degree of genetic homeostasis, but it lacks one of the main characteristics of a well-developed homeostatic system in that it offers very little possibility for artificial selection to pull the population away from its stable composition When we speak of a population as showing genetic homeostasis we usually mean that it exhibits stability of gene frequencies while still preserving genetic variability This is the state of affairs so long as the optimum is attained only, or in the main, by heterozygous genotypes In the type of set-up we have been *considering, this is a transitory stage in the passage towards* gene-fixation, but we have seen that there are reasons to expect such stages to endure a very considerable time

Further, gene-fixation and the passage to homozygosity, will be still more delayed if the environment does not remain perfectly uniform, but alters from time to time so that the selected optimum is not always the same For instance, there may be seasonal

fluctuations, such that one genotype is more suitable in summer and another in winter, and if the reproductive cycle of the individuals is short in comparison with the environmental changes, these will impede or entirely prevent the process of gene-fixation (cf Dobzhansky 1948, 1951, 1956), and thus provide a second type of explanation for the evolution of genetic homeostasis

A more far-reaching explanation of the evolution of systems which exhibit genetic homeostasis combined with the preservation of genetic variance relies on the suggestion that there may be some merit in heterozygosity as such It might be, for instance, that the existence of two different alleles at a locus gives a heterozygote a greater capacity for epigenetic adaptation to environmental fluctuations during its development Lerner (1954) in particular has emphasised this theory Although it seems most improbable that heterozygosity can be accepted as the main mechanism on which developmental buffering depends (see p 53), it certainly cannot be excluded as a factor in the make-up of

The strategy and tactics of development

The epigenetic landscape, which has been suggested as a model in terms of which development can be imagined, expresses properties which are essentially formal in nature Such value as the model possesses would be retained even if the processes by which development occurs are of quite a different nature to those from which we have in this chapter derived it We have discussed development in terms of reactions competing for substrates, or influencing one another by specific inhibitions or stimulations It is possible, however, that systems of essentially the same formal properties might be produced by processes of quite a different kind For instance, it might be suggested that development is brought about by the synthetic activity of fairly large cytoplasmic particles, built up by the *coming together in specific and definite* arrangements of a number of subunits, each manufactured by the appropriate gene, and that the distinctness of the alternative

possible paths of differentiation is to be explained by the limited number of ways in which such complex particles can, as it were, crystallise out On such a model, the difference between two courses of differentiation would ultimately depend on differences of the same kind as those between the two forms of a crystal or between the different molecular species which can be formed by combinations of atoms from the same range of elements, that is to say, on phase differences

When one remembers the high degree of orderly structure which the electron microscope is beginning to reveal not only in cytoplasmic particles but often in the optically clear cytoplasm itself, it seems almost certain that the tactical measures by which development is brought about must involve something a good deal more complex than simple interacting autocatalytic reactions

The same conclusion would seem to be suggested by the astonishing finding reported by Lewis in connection with the complex bithorax locus in *Drosophila* Here we have a short stretch of chromosome, corresponding to a group of very closely linked genes or 'pseudo-alleles', all of which affect the differentiation of the meso- and meta-thoracic imaginal buds into thorax, wings and halteres Five pseudo-alleles are now known (Lewis 1951) They lie along the chromosome in the order bithorax (*bx*), Contrabithorax (*Cbx*), Ultrabithorax (*Ubx*), bithoraxoid (*bxd*) and postbithorax (*pbx*) Now it emerges that there is some relation between the position of the allele on the chromosome and the region of animal which it affects, roughly, the sequence from *bx* to *pbx* corresponds to the passage from a more 'anterior' type of effect to a more 'posterior' one Thus *bx* tends to convert the anterior part of the metathoracic bud into the anterior part of the mesothorax, *Cbx* transforms the posterior part of the mesothorax into the posterior part of the metathorax, *Ubx* and *bxd* cause the posterior part of the metathorax to become like the posterior part of the mesothorax, while *pbx* has a similar but still more markedly 'posterior' action The fact that all the genes of the group exert 'position effects' on one another, each operating more or less effectively according as it does or does not lie in the same chromosome as another mutant allele, is an example which demonstrates

that epigenetic reactions do not always take place freely within the whole volume of the cell or even of the nucleus, but may be focused in quite small regions of it, in which case the conditions in the immediate neighbourhood of the focal region may have an important influence The nature of this localisation is still in doubt It may be that a series of pseudo-alleles, such as those of bithorax, act in sequence on a substance which diffuses gradually along the chromosome, rather as machine tools operate on a block of metal passing along a production line This is the hypothesis favoured at present by Lewis But the extraordinary correspondence between the sequence *bx*, *Ubx*, *bxd* and *pbx* and the gradually more posterior section of the metathorax which they affect, suggests that we may be dealing with epigenetic reactions mediated by structural elements which might be more comparable to the membranes seen by the electron microscope in the cytoplasm than to any type of chemical molecule

Even if development is in fact dependent on comparatively large structured elements and not on free molecules, its general formal properties will still need to be stated in terms of an approach to discrete alternative end-states along canalised paths, and the epigenetic reactions will be expressible in some system of simultaneous differential equations involving products and powers of the concentrations of the reactants The discussion given above will, then, retain its relevance to the strategy of developmental change even though we are still very largely ignorant of the exact nature of the tactical processes by which differentiation is brought about

Although we know so very little about the nature of the primary products through which the genes operate, there are certain other questions about the tactical organisation of developmental pathways which it is already worth discussing, since they may be within reach of experimental attack A survey of the types of developmental abnormality produced by mutant genes suggests that in any developmental path we can, at least roughly, distinguish between what may be called 'focal' and 'peripheral' factors The majority of mutant genes affect the latter only They cause an organ or tissue to develop abnormally while remaining

recognisably and essentially similar to its normal form For instance, all eye colour or hair colour genes leave the eyes or hair perfectly recognisable as such, the numerous genes affecting the wings of *Drosophila* nearly all cause the appearance of something which is definitely a wing, though an abnormal one, in the phenotypes of the 'rough eye' genes, not only are the eyes *obviously still eyes, but one can usually find all the cell types* such as cones, ommatidia, pigment cells, etc The epigenetic processes altered by such genes can be considered as somewhat peripheral factors in the determination of the creode On the other hand, genetic factors such as aristopedia, podoptera bithorax and a few others affect factors which are more essential, and can indeed be called focal, since when they no longer operate, the organ or tissue does not appear at all in its normal form, the cells being changed into something else

One can, of course, find cases in which such a distinction is difficult to apply For instance, a slight shift in the relative positions of the trichogen and tormogen cells in *Drosophila,* such as that produced by the gene *Stubble,* causes the macrochaetae to be thicker than normal, clearly an example of an alteration of a peripheral character But a somewhat greater shift, such as is common with *Hairless,* results in the complete conversion of what should be the bristle into an extra socket (Lees and Waddington 1942), and this might lead one to consider the geometrical relation of the two cells to be a focal factor in bristle development However, the chemical constitution of the socket is probably not very different from that of the bristle, and when one gets down to such details of developmental pathways as this it is somewhat arbitrary what one takes to be focal rather than peripheral Such difficulties are less likely to arise in connection with the broader categories of development, such as the formation of the main tissues of the body, the nervous system, muscle, etc

The existence of such borderline cases suggests that it is unlikely that any sharp distinction can be found between the types of genetic determination of peripheral and focal factors Soon after the genetic control of the branching system of developmental pathways had first been extensively discussed (Waddington 1939

1940), Mather (1943) suggested that the minor 'quantitative' variations of organs are dependent on a type of gene different from that which determines the major developmental alternatives The former he called 'polygenes', the latter 'oligogenes' I argued at the time (Waddington 1943) that the available embryological and genetical information made it impossible to sustain this distinction Since then Goldschmidt's studies of podoptera, in which numerous genes each of small effect (typical 'polygenes') co-operate to determine whether the dorsal mesothoracic bud of *Drosophila* will develop into a wing or a leg-like appendage, have provided a clearcut refutation of the idea, and there are many other examples which argue in the same sense, for example the assimilation of the bithorax phenotype by the accumulation of minor genes, which is described on p 174, or the genes affecting the skeleton of the mouse discussed by Gruneberg (1952a)

Another idea about the organisation of a creode which has come from breeding experiments is Lerner's recent suggestion that homeorhesis, i e the attainment of a relatively invariant end state, is dependent of the level of heterozygosis in the individual, animals with a large number of homozygous factors always being in some way abnormal This hypothesis was only intended to apply to comparatively minor variations from the normal, and Lerner does not appear to have considered the problems involved in the major decisions between alternative pathways, which are taken early in development But even with this limitation, it can hardly be accepted as generally true, although it may be so in particular cases (cf Dobzhansky and Levene 1955) Several authors have pointed out that inbred and highly homozygous strains are not always more variable than crossbred or heterozygous ones, although they often are (e g Mather 1953, Robertson and Reeve 1952, 1955, Tebb and Thoday 1954) From a more general point of view, a study of the development of an organ, such as the *Drosophila* wing (Waddington 1940), shows that in many phases there is a balance between various interacting processes, each of which may be affected by many non-allelic genes, and under such circumstances it is impossible to suppose that the outcome can be dependent only on the relations between

the alleles belonging to the same locus Heterozygosity cannot, therefore, be the essential condition on which homeorhesis depends This does not imply, of course, that there may not be many instances in which a heterozygote at some locus may be better buffered than either of the two corresponding homozygotes, but even if this is so, homeorhesis, as a general principle of epigenetics, must have a wider basis

The attempts to understand the organisation of a creode which we have just discussed have not led very far It is possible that more progress could be made if one returns to the consideration of what have been spoken of above as the 'focal' factors These are factors on which the various organs and tissues are existentially dependent. They may be of very different kinds For example, if for some reason the inductor of an organ does not reach the tissue competent to react to it, the organ will not appear, and we can say that a focal factor has been altered In the development of the imaginal organs of *Drosophila*, it seems likely that processes of growth during the larval period determine the way in which the epithelium of the buds becomes folded and that this in turn is a focal factor in the appearance of the final organs (cf Waddington 1942) It is easy to see how such processes of growth or of the movements leading to contact between inducer and reacting tissue, could be altered either by single genes, or by accumulations of genes each with small effects, so that no distinction between focal and peripheral factors could be made on that basis

The situation might be different if we can suppose that the focal factor sometimes takes the form of the appearance of a single essential substance or a few substances There is no doubt that some fully-differentiated cells contain very high concentrations of certain characteristic substances, for instance, muscle cells with their myosin and actin, blood corpuscles with haemoglobin, and so on Can we take it that the production of these substances is a focal factor in the creodes leading to these differentiated tissues? One would be encouraged to think so if it were to be found that the first stage in which a developmental pathway of this kind diverged from the main stream, and acquired its own character, involves the appearance of the characteristic substance, which

could then be regarded as a 'histogenetic key-substance', around which all later differentiation is organised (Fig 6) Experiments using immunological methods have already given some evidence that this may be so in some cases For instance, ten Cate and van Doorenmaalen (1950) found that in the chick the lens epithelium acquires the ability to react with antisera prepared against differentiated lenses very soon after it first shows any sign of thickening Similarly Ebert (1950, 1954) showed that other early embryonic tissues of the chick (neural plate, mesoderm, etc.) react, soon after their first appearance, with antisera against their later derivatives The same author found that cardiac myosin (or something which reacts with antisera against myosin) is present in the chick embryo even before the position of the heart is fixed. At this precocious stage, the myosin is distributed over a greater area than that which will eventually become the heart One of the earliest signs of the initiation of a definite heart creode is an increase in the concentration of cardiac myosin in the presumptive heart regions and its disappearance elsewhere, and this is shortly followed by the appearance of cardiac actin in the same areas

In these cases, the substances characteristic of the adult tissues put in an appearance very soon after the corresponding region of the embryo takes the first steps along the appropriate developmental path. Such a situation is favourable towards the hypothesis that the lens protein, nerve protein or heart myosin are histogenetic key-substances, and that it is their initial appearance which determines the later developmental fate of the rudiment in which they are formed It is, of course, by no means clear that this hypothesis will always be true For instance, in Amphibia the embryonic blood cells are formed from a group of mesoderm cells (the 'blood island') which is determined, in the ventral side of the neurula, some time before it is possible to demonstrate the presence [illegible]

is possible that the tendency to develop into the latter does not become determined till the time at which haemoglobin begins to be formed. Thus this case may not, after all, contradict the

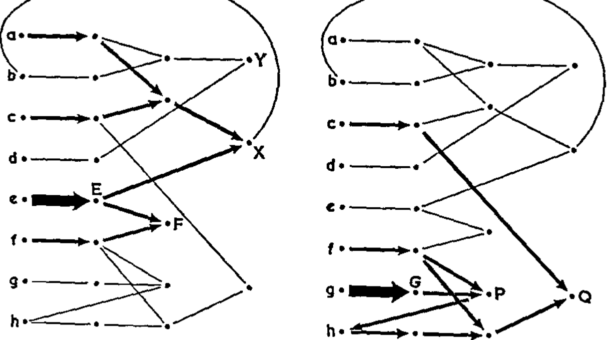


FIGURE 6

The organisation of a developmental path round a focal reaction Both halves of the diagram show the same set of inter-related reactions On the left side, a high activity of the process *e* to *E* brings into play a number of side reactions, leading to the formation of substances such as *F* and *X*, the latter will soon begin to exert a feedback action on the *b* to *Y* sequence On the right the focal activity is in the *g* to *G* process and this activates quite a different set of secondary reactions, leading to substances such as *P* and Q

hypothesis which links determination to the production of a histogenetic key-substance

We still know so little about the nature of the chemical differences between cells belonging to different tissues that we have no firm ground to decide whether a histogenetic key is likely to be a single substance or a group of substances The latter supposition is, however, the more general one, and in the case of the chick heart studied by Ebert, both myosin and actin are produced in very early stages of the creode

The substances which might be expected to form components of histogenetic keys are primarily proteins The manner in which genes control protein formation is probably somewhat unlike the picture of gene action which usually takes the central place in discussions of biochemical genetics Most of our knowledge of the genetic control of substances is concerned either with molecules which are clearly peripheral factors in development (e g eye colours, flower or hair pigments, etc) or with metabolites of relatively low molecular weight (such as those involved in the metabolic blocks in micro-organisms) From these studies, one derives a picture of genes operating in sequence, one or more of them affecting each step in a reaction series There is, however, so far as I know, no example demonstrating the existence of such a sequence of steps in the synthesis of a specific protein. In the best-understood instances of the genetic control of proteins, such as that of the haemoglobins in sickle-cell anaemia and similar conditions, change in a single locus alters the whole specificity without apparently halting the synthesis at an intermediate stage (cf. Haldane 1954) Even though it is probable that several genes are acting, they seem to operate concurrently rather than sequentially

A similar suggestion arises from studies such as those of Spiegelman on adaptive enzyme formation He found that a single unnatural amino-acid could reduce the incorporation of any other amino-acid into the protein being formed, and argued from this that these enzymes are produced direct from a pool of free amino-acids rather than by sequential modification of more elaborate precursors There is very similar evidence both in chick and amphibian embryos, suggesting that the proteins are produced

from a free amino-acid pool, that is by concurrent and not sequential processes (Waddington and Sirlin 1954, Feldman and Waddington 1955) It seems rather likely, therefore, that, if there are such things as histogenetic key proteins, they are formed by the concurrent action of relatively small numbers of genes (It may be that some sequential modification of them occurs in later stages of development, for instance when the muscle cells become regionally specific as tail or trunk muscle, etc.)

It may then be useful to envisage a creode, at least in its early stages, as being organised around the concurrent synthesis, controlled by comparatively few genes, of one or more histogenetic key substances As this process gets under way, it will come to dominate the whole anabolism of the cell, superseding other processes which may originally have been of equal importance, and as it were picking up and sweeping along with it an entourage of more peripheral processes, such as the production of pigments or similar inessential cell constituents

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Note to p 47

In a paper read to the Cold Spring Harbor symposium in 1957, Lewontin discussed in more detail his understanding of the concept of homeostasis It was clear that he takes cognisance of all the various types of phenomena which in this book are described as genetic or physiological homeostasis or developmental homeorhesis, but he refers to them in other ways reserving the word homeostasis for the holding constant (or, it should be, for the maximising) of *fitness* Thus when he speaks of developmental or genetic 'homeostasis', he has [illegible]

used consistently in this sense, but then, in my opinion, loses all connection with its previous uses For instance, a state of hibernation would be said to be 'physiologically homeostatic' because it subserves fitness, although it does so by an alteration in the total physiological state of the organism

CHAPTER 3

SELECTION OF, FOR AND BY

DURING THE the last 30 years the study of evolution has been most actively and successfully taken up from a new point of view, that of genetics This attack has been made from two sides, by mathematical theorists on the one hand and by experimental naturalists on the other The advances made have been so striking that they may even be taken to have reached their goal with some degree of finality Thus in recent years there have appeared a whole series of treatises, by Dobzhansky, Simpson, Mayr, Rensch, Huxley, Cuénot and others, almost any of which might have taken the proud title of Huxley's work—*Evolution, the Modern Synthesis* Among biologists, it has been primarily those with an embryological background, who have continued to pose questions Goldschmidt (1940, 1951) with his ideas of the 'unbridgeable gap' and systemic mutations, Schmalhausen (1949) with his notion of stabilising selection, and Dalcq, one of whose recent articles (1951) has the slightly ironical title 'Le problème de l'evolution est-il pres d'être résolu?'.

In what precisely have the recent advances consisted? One can probably take the mathematical theory as being the earliest aspect of the truly modern outlook to be developed Its origin may perhaps be seen in a series of papers by Haldane, begun in 1924, and it soon became enshrined in three major works, by Fisher 1930, Wright 1931 and Haldane 1932 Examining it after this lapse of time one finds, unexpectedly, that it did not achieve either of the two results which one normally expects from a mathematical theory It has not, in the first place, led to any noteworthy quantitative statements about evolution. The formulae involve parameters of selective advantage, effective population size, migration and mutation rates, etc., most of which are still too inaccurately known to enable quantitative predictions to be

made or verified But even when this is not possible, a mathematical treatment may reveal *new types of relation and of process*, and thus provide a more flexible theory, capable of explaining phenomena which were previously obscure It is doubtful how far the mathematical theory of evolution can be said to have done this Very few qualitatively new ideas have emerged from it.

Some of the ideas which are often credited to it were in fact well known before it was developed. For example, a problem which greatly puzzled Darwin was how hereditary variation could remain in existence in a population from generation to generation, if, as he thought, the hereditary factors from the two parents blended in the offspring, genetic variance would disappear at a rapid rate Many authors at the present time, even professional geneticists such as Darlington (1953, p 69) and Huxley (1942 p 55), as well as other well-read persons such as Irvine (1953) suggest that this problem was not solved until 1930 when, in Darlington's words, 'R A Fisher, in his illuminating work, *The Genetical Theory of Natural Selection*, went into the whole Darwinian theory with the full rigour of his analytic methods' Actually the basic point at issue is not a mathematical one at all The variance is preserved simply because hereditary factors, or genes, do not blend as Darwin thought, but preserve their integrity and segregate The relevance of this fact to the matter at issue had been perfectly clearly recognised by Bateson at least as early as 1909 when he wrote (*Mendel's Principles of Heredity*, p 288) 'The notion that a character once appearing in an individual is in danger of obliteration by the intercrossing of that individual with others lacking that character proves to be unreal, because in so far as the character depends on factors which segregate, no obliteration takes place'

Wright's theory of drift (p 82) has, perhaps, the most convincing claim to be a quite fresh and novel outcome of the application of mathematical thought to evolutionary problems, but several authorities express grave doubts whether it actually plays any important part under natural conditions

What then gave the mathematical theory its undoubtedly immense importance and prestige? They seem to have had two

grounds In the first place it was shown that the ordinary, well-known Mendelian genes would respond to the processes of natural selection and could thus be the entities on whose variation evolution depends, and secondly, it was demonstrated that continuous variation, which is much commoner in nature, does not differ essentially in its biological causation from discontinuous, but like it depends on genes Thus the outcome of the mathematical theory was, in the main, to inspire confidence in the efficiency of the process of natural selection and in the justice of applying this type of argument also to the realm of continuous variation

At the same time as the mathematical theory of natural selection was being elaborated, an extensive and penetrating series of researches have been made on evolutionary phenomena in nature A Russian school, now widely scattered throughout the world, has been very active in this field, one remembers particularly the names of Tsetverikov, Timofeeff-Ressovsky, Dubinin and Dobzhansky, but many others, also of other nationalities, have contributed The first result of these studies was the revelation of a wealth of genetic variation, both genic and chromosomal, which could not be discovered by a mere inspection of the adult individuals in a population Much of the genic variation is carried in the form of heterozygous recessives, while the chromosome variation can only be detected by special cytological or genetical techniques Both kinds can, however, become available for evolution Moreover, investigations of local races and nearly related species have shown that they frequently differ from one another in ways which are essentially similar to those which cause variation within a single interbreeding population The materials are therefore available to support the hypothesis that evolution has been brought about by natural selection acting on genetic variability of this kind Further, in a certain number of cases, though unfortunately not in very many, the actual occurrence of natural selection can be demonstrated

The general result of these two types of approach to the problems of evolution would appear to be that we have gained considerable grounds for confidence in the reality and effectiveness of evolutionary mechanisms of the kind contemplated in the modern

theory of natural selection We know that these mechanisms can, and in some instances do, operate on the variation which is plentifully produced by processes of 'random' mutation, both chromosomal and genic It would in fact appear that the known processes of selection are powerful and flexible enough to account for the observed evolutionary changes, provided that the material they have to work on—the available variability—can be accepted as adequate in kind It is in connection with the nature of the available variation that modern theories of evolution are most in need of expansion.

There seem to be three major fields in which inquiry is called for One is the problem of adaptation Is it really sufficient to suppose that the extraordinarily precise fitting of an animal into its ecological niche is due solely to the selection of random variations? We know of many cases in which the environment of a particular locality—for instance, a mountain range or a swamp—will produce in individuals from some other region non-hereditary modifications which are strikingly similar to aberrant forms which in the local population have become genetically determined Are we to suppose that such parallelism is completely beside the point, and that evolution of a local genetically fixed ectotype has been based on mutations which have occurred at random and are thus quite unconnected with the direct developmental effects of the environment? We shall discuss this question at length in Chapter 5

A second context in which the adequacy of the present theory has been questioned is that of the nature of the differences between species or species groups Goldschmidt (1940), in particular has argued that in nature certain fairly large groups of forms differ from each other in some way which is not directly comparable to the manner in which the local races of a single species or the species of a single species group, are related He has spoken of an 'unbridgeable gap' between such major groupings, and has suggested that the gaps arise *by some special type of 'systemic' mutation*. His attempts to suggest a mechanism for mutations of this kind have not appeared to most other geneticists to be very convincing, and this has perhaps somewhat obscured the cogency

of his arguments for the existence of the unbridgeable gaps themselves

But there have recently been some developments which, perhaps, make the occurrence of a complete reshuffling of the genotype appear not quite so inexplicable as it did a few years ago The transduction by a phage particle of a linked group of genes from one bacterium into another, where it joins up with the genotype of the host cell, and again the rapidly accumulating evidence of 'conversion' affecting considerable groups of linked factors,

and become the definitive genes of the new chromosome And if this, or something like it, occurs and moreover sometimes goes wrong so that the 'incorrect' (transduced or converted) section becomes incorporated into the nascent chromosome, then it is not too difficult to conceive that very occasionally there may be a more radical misbehaviour of the pre-genes, resulting in them joining up in a quite novel order At present it is only as a hardly supported speculation that one can advance such an idea about a mechanism for a 'systemic mutation' But even so, it seems likely that we are very far from having heard the last word about the nature of the variation on which Goldschmidt's unbridgeable gaps depend

As a third major question-mark in evolutionary theory there remains a series of questions which arise mainly from palaeontology Many students of the past history of living organisms have been tempted to use phrases such as 'racial juvenility or senescence', 'evolutionary stability', 'evolutionary potential' and the like, which are supposed to apply to the types of evolutionary change occurring in large groups of related organisms over very long periods of time The conventional genetical theory of the present day could, perhaps, begin to approach such matters from the consideration that it is known that mutation rates are to some extent under genetical control and can therefore be assumed to have different values in different groups But it seems probable that mutation always provides far more variation than is utilised

for actual evolutionary change, and it is therefore rather implausible to suggest that the phenomena which have been referred to by terms such as 'evolutionary potential' can be interpreted in terms of mutation rates In authoritative recent discussions, such as those of Simpson (1944, 1949), for instance, some of the phenomena in question, such as differing rates of evolution, are left unexplained, while the reality of others is called in question For instance, many palaeontologists in the past described examples of lineages which were alleged to have followed one consistent trend of change throughout long periods of evolution Simpson is at pains to minimize the importance of such phenomena, and it is probably true that in the heyday of the fashion for such interpretations, many examples were quoted (the evolution of the horse is a well-known instance) which will not bear examination. But the unprejudiced student is likely to derive the impression that the failure of present theory to provide any plausible explanation for such occurrences has played a not unimportant part in weighting the scales against an acceptance of their real existence It would certainly seem that in this field again the adequacy of modern theory may be doubted

Modes of selection

The current theory of evolution attributes this process to the natural selection of random variations In a certain sense this phrase probably conveys the essential truth about the nature of the processes concerned It is, however, only too easy to accept it as an adequate answer to our questions, whereas it should really be taken as an introduction to a series of problems As it stands, the first part of it, 'natural selection', is a tautology, and the second part, 'random variation', an equivocation

The meaning of natural selection can be epigrammatically summarised as 'the survival of the fittest' Here 'survival' does not, of course, mean the bodily endurance of a single individual, outliving Methuselah It implies, in its present-day interpretation, perpetuation as a source for future generations That individual 'survives' best which leaves most offspring Again, to speak of an animal as 'fittest' does not necessarily imply that it is strongest, or

most healthy, or would win a beauty competition Essentially it denotes nothing more than leaving most offspring The general principle of natural selection, in fact, merely amounts to the statement that the individuals which leave most offspring are those which leave most offspring It is a tautology It is only when we penetrate beyond the field of generalities, to consider what different kinds of selection might be expected to occur, that we pass out of the sphere of empty truisms into the region where empirical scientific investigation is possible

In the mathematical theory of evolution natural selection is normally treated simply as a process which brings about changes in the frequencies of the genes in a population Selective coefficients are attached to the alleles present in the population Such a procedure does indeed summarise the essential action of natural selection, since it is only by alterations in gene frequency that hereditary changes occur as generation succeeds generation However, in deriving the theory solely from the abstract logical nature of the end result of selection, one runs a considerable danger of omitting to consider features of the process which are important in determining the nature of the changes which happen Selection usually does not operate directly on the genes It depends on the ability of *organisms* to leave offspring It is the phenotype throughout its development which is exposed to the rigours of the environment, which falls a prey to disease, the inclemency of natural conditions, or predation, which is successful or unsuccessful in mating, which is fertile or infertile That is at least the usual situation, but processes of natural selection of rather diverse kinds have been invoked by various authors dealing with different situations, and it would seem advisable to distinguish a number of different categories of selective processes

There has, in fact, been at least one suggestion that processes of selection may sometimes occur at the level of the gene itself Lima

disposition of granules or chromomeres He suggests that if at any locus on a chromosome a mutation occurred to a gene, whose

physiological properties did not fit into the general chromosome pattern in these respects, there might be a direct selection against it on that account Again, we know, both in maize (McClintock 1951) and *Drosophila* (Mampell 1946), of 'genes' (or small chromosome aberrations) which cause a tendency for abnormally high rates of mutation of other genes, particularly those in their immediate neighbourhood. It is reasonable to suppose that there would usually be a selective pressure against the appearance of such mutator genes Both these cases could perhaps be considered to provide examples of a category of selection which operates, not on the phenotypic results of the developmental activities of genes, but directly on the genotype itself

These examples are, however, not very convincing Lima da Faria's is purely hypothetical, there is no direct evidence that mutations ever do produce genes which break up the patterns of stainability which he has described, or that if they did so, there would be a selective pressure against such new genes And the harmful effects of the mutator genes can quite well be regarded as brought about through the developmental abnormalities produced by the genes which they cause to mutate

The most usual and widely recognised type of selection is, as was pointed out above, a process which operates on the phenotypes produced by the developmental activity of the genes We *have here to distinguish the number of sub-categories There is* first a distinction to be made between what may be called individual selection and progeny selection Consider an organism carrying a genotype *p* which develops into a phenotype *P* Individual selection occurs when the frequency of the genes of *p* in future generations depends on the character of *P*, progeny selection, when it depends on the character of the progeny of *P*

We will discuss later some examples of progeny selection Before doing so there is another distinction to be made within the general class of individual selection. This may be divided into selection of the first or higher orders By a first order selection we mean the selection for the presence or absence of some particular gene *A* in the genotype *P* This first order individual selection is the type which has been most fully treated in the mathematical

development of the theory For instance, we may consider a population heterozygous at a locus *Aa*, and suppose that at equilibrium it contains $U^2AA, 2UAa, 1aa$, which after selection becomes reduced to U^2AA $2UAa$ $(1 - k)aa$, and from this as a basis a whole elaborate mathematical theory may be developed.

Modern evolutionary theory, however, is not content with such first order selection of the gene *A* itself, but freely invokes processes of the selection of the modifiers of *A* That is to say, the selective coefficients are attached, not simply to the alleles of the *A* locus itself, but to the combinations between these alleles and certain other genes which modify their phenotypic expression. Such processes of selection must be considered to belong to a second, or possibly in some cases even a higher order They have been studied in the laboratory at least since the days of Marshall and Muller (1917) Their vogue in consideration of the evolution of wild populations may perhaps be dated from Fisher's paper of 1928 on the evolution of dominance In this he argued that if during the evolutionary history of a population a gene frequently mutates to a form which is partly dominant and deleterious in its effects, there will be a selection of modifiers which reduce the dominant expression of the gene in the heterozygous form. Now unless we suppose these modifiers also to be selected for some other reason, the selective pressure will only affect them when they are combined with the rare new mutatnt The frequency of occurrence of the favourable combination between the mutant and modifiers will, of course, be much lower than the total frequency of the mutant, and it is for this reason that the selection must be considered as a second order one I do not wish to call in question the reality and the importance of such second order processes In point of fact one gets the impression, at least from experience with laboratory stocks that they occur more readily and more completely than might perhaps have been expected The mathematical theory of the progress of selection in such cases, however, would seem to demand more attention than has as yet been paid to it

Haldane has recently (1956) discussed one such case and come to rather unexpected conclusions about it. Kettlewell (1956) has

shown that the dark form (*carbonaria*) of the Pepper Moth (*Biston betularia*) has, in industrial areas, a considerable selective advantage over the normal pale form, and has indeed largely replaced it The dark form is dominant, and there is evidence that during the replacement of the pale by the dark type, modifiers have been selected which have greatly intensified this dominance, which has now become almost complete in the regions in which *carbonaria* makes up the great majority of the population Haldane shows that, if the advantage of the dark form over the light has remained approximately the same, there can never have existed, during the whole period when the spread of *carbonaria* was taking place, a sufficiently large number of heterozygotes to provide a plausible basis for the supposed second order selection by which the dominance is alleged to have been intensified He concludes that the problem of how this selection may have operated is not yet solved, but suggests that possibly in the past the selection coefficients were different, and were of a kind (e g with the heterozygote favoured, not for its colour but for some other aspect of fitness) which caused heterozygotes to be much commoner than the straightforward selection of the dark forms would require

We will now return to the question of progeny selection Perhaps the most important context in which this process has been postulated is in connection with the evolution of reproductive isolation between diverging local populations or sub-species Suppose that in two contiguous geographical areas we have two populations *A* and *B* each adapted to its own habitat Then if the hybrids between *A* and *B* are less well adapted to either habitat than the original races, it is suggested that we shall have a selective pressure against the willingness of *A* individuals to mate with *B*, or vice versa, and that this will lead to the formation of a reproductive barrier between the two populations Now this postulated selection operates between the different individuals of population *A*, not on the basis of their own fitness (i e on the number of offspring they leave), but on the basis of the fitness of their progeny (i e the number of grandchildren they produce) Again there is little doubt that such types of selection may occur.

Indeed, in my laboratory we have carried out such selection artificially. Populations of *Drosophila* each labelled with a recessive marker gene were mixed and allowed to mate at random, but of their offspring only those were allowed to breed which had resulted from a mating of like with like, the progeny of cross-matings being eliminated. After a number of generations a considerable, though not absolute, barrier to cross-mating had been built up (Fig. 7) (Knight, Robertson and Waddington 1956, see also Wallace 1950) Again, the mathematical theory of the process must be considerably different from that of first order individual selection and has still been very inadequately explored.

There is still a further type of selection frequently invoked by recent writers on evolution which is even further removed from the simple first order individual selection It is a selection which operates not between individuals but between populations. There can, of course, be a relatively simple selection between populations if, for instance, a local race, which has become particularly well adapted and efficient in one region moves into another area occupied by a less highly evolved group, which it would then succeed in eliminating. Selection in this case, however, would operate [illegible]
of one p[illegible]
merely [illegible]
migration. The competition between populations which is now being referred to is of a different type. It is a competition through the ability to respond to new selective pressures.

The most thoroughgoing postulation of such processes has been by Darlington (1939), who has discussed the evolutionary consequences of various types of genetic system He points out how certain reproductive systems, such as apogamy or self-fertilisation, may be highly efficient in preserving a favourable genetic constitution of a population in a relatively uniform environment, but will tend to deprive that population of the capacity to become genetically modified when new selective demands are made on it. It will therefore usually be an evolutionary dead end Other systems, such as normal cross-fertilisation, will be more flexible, and populations possessing them will therefore have a

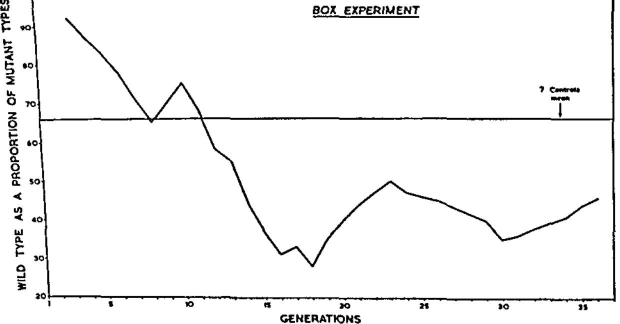


FIGURE 7

Selection for sexual isolation in *Drosophila melanogaster* Homozygous mutants of two types (ebony and vestigial) were allowed to mate at random Matings of two ebonies give ebony offspring, and of two vestigials give vestigial, but cross-matings give wild-type When the population was carried on by selecting as parents only the two mutant types, the frequency of wild-type offspring gradually fell, that is, there was an increasing tendency for matings of like with like, and a reduction in the proportion of cross-matings (From Knight, Robertson and Waddington 1956.)

long-term evolutionary advantage The same type of idea is probably what is meant by Dobzhansky (1951) when he speaks of certain populations which contain a great deal of hidden hereditary variation in a heterozygous form as being 'better adapted' than others which are genetically more uniform Such processes of selection between populations on the basis of their ability to respond to new environmental demands are, of course, highly hypothetical and hardly susceptible as yet of laboratory experimental testing They might perhaps be referred to as metaselection—something going beyond selection proper as metaphysics goes beyond physics

A type of selection which is in some way intermediate between metaselection and progeny selection was discussed by Haldane, even with some mathematical treatment, as long ago as 1932 He considered the case of a gene which caused the individual exhibiting it to leave fewer offspring than normal, but to behave in such a manner that the population to which it belonged increased in numbers He showed that there are some situations in which there would be an increase in frequency of such genes, which, as he pointed out, produce altruistic behaviour, but he considered that it was difficult to see how normal selective processes could bring into being a population entirely composed of altruists

The types of selection which have been discussed above are distinguished from one another according to the nature of the entity on which they act on individuals, on their progeny in later generations, or on populations There is another, and in some ways even more important, way of classifying selection, in relation to the type of effect which it produces It is usual to recognise two such categories

Firstly, selection may be progressive, when it leads to change in the phenotype of the population, producing alteration in gene [illegible]

push the population simultaneously in two different directions this, which he calls 'disruptive selection', can be adequately

regarded as a sub-type of the more usual unidirectional progressive selection

Secondly, selection may act in a stabilising manner to preserve the original phenotypic character by eliminating new deleterious mutations It has long been realised that this type of stabilising action is perhaps the most general and usual function of natural selection It is only comparatively recently, however, that attention has been drawn to the fact that stabilising selection may operate in two and probably three different ways In the first place, a population inhabiting a uniform environment exhibits a phenomenon which has been called 'genetic homeostasis' (Lerner 1954) The frequency with which any particular gene is present in the population settles down to an equilibrium which is determined by the pressures of selection, mutation, migration, etc If some slight change from the equilibrium position is brought about (and if it does not set in operation a drift-reinforcing mechanism of the kind mentioned on p 87), then these pressures will bring the frequency back to the equilibrium, the selection pressure being usually the most effective agent in this return This type of selection we may call 'normalising' It usually operates by a first order individual process Stabilising selection may, however, operate in another manner Some years ago I pointed out (Waddington 1940, 1942) that the phenotypic constancy and uniformity of a population could be ensured by selection in favour of genotypes which control developmental systems which are highly canalised and therefore not very responsive either to abnormalities in the environment or to new gene mutations of a minor character Such a type of selection may be called 'stabilising selection (*sensu stricto*)', or better, 'canalising selection' Finally, it has been pointed out (p 41), that selection might operate against the 'noisiness' which causes strictly homologous organs sometimes to diverge slightly in their development It was suggested that this *might* be called 'selection for repeatability' It probably plays relatively little part in evolution, and from our present point of view at least is of much less importance than the two major categories of normalising and canalising selection

Before the train of thought which originally led to the

formulation of these ideas had proceeded very far it was unfortunately broken off by some of the more pressing business that demanded one's attention at that time (in point of fact, an ecological study of predation between aircraft, U-boats and ships became for a time more engrossing even than Nature red in tooth and claw) At the same period very similar ideas were being discussed by Schmalhausen (cf 1941) in various publications, most of which appeared in Russian. More recently he has given a full discussion of stabilising selection in his book *Factors of Evolution* which was translated into English in 1949 Schmalhausen undoubtedly describes a process similar to that which I have called canalising selection or stabilising selection (*sensu stricto*). He does not seem to me, however, to distinguish it at all clearly from the other type of stabilising selection which I have suggested we might refer to more precisely as normalising selection. It may be as well therefore to discuss the two types in rather greater detail and then consider in a preliminary way how a population subjected to them will respond to a change in environment.

It will be as well to start with an extremely simple numerical example which exhibits the basic elements of normalising and canalising selection. Consider a non- [illegible]

character, in the proportions $a = 0$, $A = 5$, $b = 5$, $B = 10$ If the population is mating at random, the frequencies of the various genotypes will be as shown on the left of Table 1, and the dosage

TABLE I

		256 CC, 512 Cc		256 cc	
Frequency of genotypes	*Gene dosage*	*Phenotypic value*	*Viability*	*Phenotypic value*	*Viability*
9 *aabb*	10	10	0	15	½
6 *aabB*, 54 *aAbb*	15	15	½	17½	¾
1 *aaBB*, 36 *aAbB*, 81 *AAbb*	20	20	1	20	1
6 *aABB*, 54 *AAbB*	25	25	½	22½	¾
9 *AABB*	30	30	0	25	½

of gene-activity corresponding to these genotypes will be as in the

first column Let us suppose further that there is another segregating locus *Cc*, which affects the sensitivity of the epigenetic system to the disturbance produced by the action of the *Aa* and *Bb* loci We will take it that the allele *C* is completely *dominant*, and that in *CC* and *Cc* individuals a given gene-dosage becomes manifested as a phenotype which can be assigned the same index as that of the

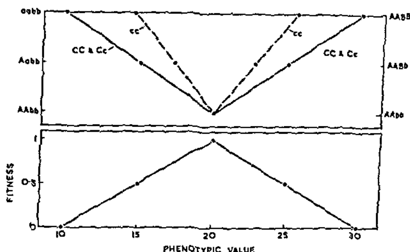


FIGURE 8

Diagram of some hypothetical gene-compounds The measurement of some phenotypic character is plotted horizontally The lower graph shows the fitness associated with variations in the character (a 'fitness cross-section', p 117) The upper part shows the phenotypic effects of certain combinations of alleles *Aa*, *Bb*, and *Cc* (see text)

the frequency of the allele *c* to be 50 per cent Finally, we assume that the phenotypic value of 20 is the optimum for the environmental conditions prevailing, and that deviations from this are less viable in the proportions shown in columns 3 and 5 Multiplying the frequencies of the genotypes by the viability factors in these columns, it is easy to show that the *CC* and *Cc* sub-population will contribute 3 × 178 individuals to the next generation,

among which the frequency of the *a* allele will be 74/356, and that of *B* will be the same. Similarly, the *cc* sub-population will contribute 217 individuals, among which the frequency of *a* will be 101/434, and of *B* the same. Thus the frequencies of the two rare extremely-acting genes *a* and *B* will in one generation change from the original values of 25 per cent to $(3 \times 74 + 101)/(3 \times 356 + 434) = 21.6$ per cent. This is an effect of normalising selection, which is tending to reduce the genetic variability by eliminating alleles with extreme degrees of activity. Again the *frequency of the allele c will have changed from* 50 per cent to $(217 + 178)/(217 + 3 \times 178) = 52.7$ per cent. This is the effect of canalising selection, which is favouring the allele which renders the epigenetic system less susceptible to the influence of abnormal intensities in the action of the *Aa* and *Bb* loci.

Very few experiments have been done which have attempted to uncover situations similar to the theoretical model just discussed. Falconer and Robertson (1956) compared the results of carrying on a population of mice, in the first place by selecting as parents those individuals with weights nearest the mean, and in the second by mating together the opposite extremes, but the results were ambiguous and showed little definite effect on the genetic variance. Falconer (1957) has more recently described the result of selection for the mean number of bristles on two abdominal segments of *Drosophila*. This was 'stabilising selection' in the broad sense, or 'central selection' as he calls it to avoid confusion with the more precise meaning of that term. Again the results were negative, in the sense that little reduction in variance occurred. But the system was not an appropriate one, since there is very little environmental variance in the character, and hence one would expect there to be little genetic variance in the strength of canalisation, alleles comparable to the *Cc* in our model seem not to have been present. There was some variance in 'noisiness', that is in the difference between the two segments, but it turned out that in fact the selection which was exercised did not impinge on this, so it is not surprising that the 'developmental stability' in this sense also remained unchanged.

With this elementary model as a background, we can now

consider a situation in which the epigenetic system is subjected to unusual environmental stresses as well as to variations in gene activity Fig 9 is intended to show, in diagrammatic form, how

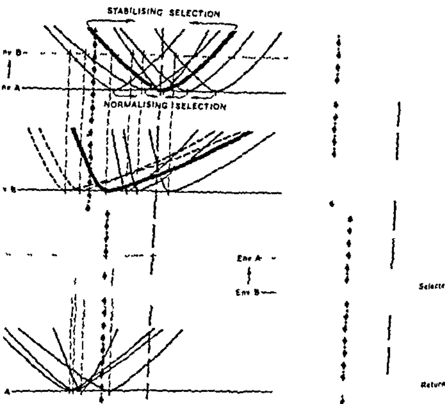


FIGURE 9
Normalising and canalising (stabilising) selection and the effects of change of environment (see text) (From Waddington 1948*a*)

these two types of selection operate At the top left, each V-shaped curve represents one individual in a population The vertical axis corresponds to some environmental variable, and the horizontal to the measurement of some character of the adult Consider the curve drawn with a thick line It is intended to indicate that in environment *A*, the genotype of that individual develops into an adult in which the character in question has the value corresponding to the dot-dashed vertical line (which is in

this instance the average value for the whole population) If the environment is altered from the normal, either in a positive sense (on the left side of the dot-dashed line) or a negative sense (on the right) the value of the adult character produced by that genotype will be given by the intercept of the new environmental value with the thick curve This curve therefore represents the canalisation cross-section of that particular genotype It is shown here as a smooth curve, with no indication of threshold phenomena, but it may presumably take very different forms in different cases

A few other genotypes in the population are shown in the other thin-lined curves It will be noted that their adult values in environment *A* are scattered around the average value with a frequency distribution which is shown in diagrammatic form in the small drawing to the right in the upper line It may also be seen that the forms of the developmental canals are represented as different in different cases

In this diagram, normalising selection appears as a tendency for the apices of the V-shaped curves to move towards the average value shown by the dot-dashed vertical line, or, what amounts to the same thing, for a diminution of the variability indicated in the frequency diagram on the right Canalising selection consists in a tendency for the upper parts of the two limbs of each V-shaped curve to be pinched together, so that the angle of the apex becomes more acute

Suppose now that the environmental changes, say to the value indicated by the line 'Env B' The V-shaped curves will be transformed into shapes something like those shown in the second drawing on the left side Selection, both normalising and canalising, will now operate on this population of environmentally affected animals It will produce results such as those shown in the third drawing in the left column, a normalisation of the population around a new average value appropriate to the new environment, and the establishment of genotypes which effectively canalise the new optimum value of the adult measurement

Let us now consider what may happen if the environment is changed back again to the condition *A* which it had at first The

effect which the change *A* to *B* originally had will not be completely reversed, since we have supposed that some canalisation of the new developmental paths has already taken place Thus the population will be shifted back, not to the condition indicated in the upper left drawing, but only *to that shown in the lower left.* The phenotypic condition indicated by the shift of the V-shaped curves to the left between the first and second drawings was originally produced simply by the substitution of environment *B* for environment *A*, it was an 'acquired character' in the conventional sense But we see that it is now to some extent retained even when the environmental conditions return to their original state, and it can therefore be considered to have become in some degree hereditary. (Naturally no character can ever be completely environmental in origin, nor yet completely hereditary, since both genotype and environment are necessary components of all development) *We might speak of this phenomenon as* 'genetic assimulation of an acquired character' The genotype has become altered so that, in a certain environment, it now produces a type of adult, which, before the genetic alteration, was brought into being only as a response to an environmental stimulus

The situation is particularly clear when threshold phenomena are involved. Suppose we are interested in a character which only appears when the value of some component of the phenotype, indicated by the apex of a V-shaped curve, falls to the left of the vertical line of circles-and-dashes In the original population (upper row of drawings) the character will be completely absent. *After change to environment B*, a few will appear (second row of drawings) If the character is selected for in environment *B* normalisation and canalisation will take place around values well to the left of the threshold, and the threshold itself will probably be lowered, i e shifted to the right in the diagram *We may* eventually find that on return to environment *A*, a considerable fraction, or even the whole, of the population continues to show the 'acquired character', which was at first quite absent from it A further consideration of such evolutionary processes is given in Chapter 5

The concept of canalising selection is closely allied to another

rather speculative notion, which I do not remember to have seen discussed, but which may sometimes be valuable to bear in mind The forms and functional systems of animals as they evolve in nature always preserve a quality which we commonly refer to by such words as 'organisation' or 'integration' This is, of course, produced by the epigenetic system, and canalising selection helps to bring it into being Some mutations may be rejected, not merely because they break down the particular canalised system which is most valuable under the particular circumstances in which the population is living at the moment, but because their action is difficult to accommodate in any well-organised course of development

An example may make the meaning of this clearer There are several genes known which affect the segmentation of the legs in *Drosophila* (Fig 10) Some of them, such as *dachs* and *four-jointed* cause the appearance of tarsi which have four joints instead of the normal five, but these shortened legs are relatively well-formed, constant in the proportion and number of their segments, and in fact would only require some slight improvement of the buffering system to be a potentially useful acquisition to the species if some environmental circumstance favoured a shortening of the legs Some other genes, on the other hand, such as *aristopedia, eyeless-dominant, dachsous, rotund*, etc , produce legs which are much less uniform from one individual to another, or even as between the different segments of the same fly, and which have irregular swellings and knobs which one would instinctively tend to consider misshapen. Such judgements must, of course, be accepted only with great caution, but one could feel confident that, in order to evolve any satisfactorily canalised system in which these genes were incorporated, a very considerable modification of the buffering system would be necessary

To find a name for the type of selection to which such genes would be subject, one might go back to the notion of archetypes, which was the central focus in the thinking of Goethe and the German *Naturphilosophen* of the eighteenth century, the idea, that is, that there are only a certain number of basic patterns which organic forms can assume The re-employment of their

language does not, of course, imply any acceptance of the structure of theory for which they used it. One can, however, designate as 'archetypal selection' the process by which genes are favoured or rejected according as they do, or do not, produce effects which are potentially canalisable into forms which might be valuable under some circumstances or other

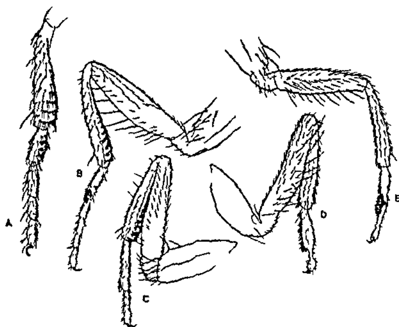


FIGURE 10
Legs of Drosophila A is a leg of *dachs* in which the number of tarsal segments is reduced to four, but their arrangement is orderly (magnification slightly higher than that of other figures)

The idea that natural selection operates, to a large extent, in a 'normalising' manner which preserves the genetic *status quo* in a population, originally arose at a time when one thought of a natural population as consisting mainly of 'wild types', all of which had essentially the same genotype apart from the existence within them of some rare recessives The 'genetic

homeostasis' of the population then consisted in a tendency for abnormal genotypes to be eliminatded, so that after a genetic disturbance (e g some generations of artificial selection) the population, if left to itself, would return to the situation in which once again all individuals had the same standard wild type genotype Nowadays, however, we do not think that the individuals in a wild population are more or less homozygous 'wild types' On the contrary, we think they are highly heterozygous, which implies, of course, that they cannot all be identical in genotype This makes the concept of normalising selection, or genetic homeostasis, somewhat less easy to formulate

Lerner (1954) conceives of genetic homeostasis as, in the main, brought about by a tendency for natural selection to eliminate individual [illegible] thus to restore [illegible] view implies that individuals which fall outside the normal phenotypic range of the population are likely to be more homozygous than normal, thus artificial selection will usually tend to produce homozygotes But such a view is by no means necessary It probably derives much of its plausibility from an unconscious assumption that most loci exist in the population only in two alleles Wherever the matter has been tested, however, many very slightly different alleles ('iso-alleles') have been detected in wild populations (e g Spencer 1944, Stern and Schaeffer 1943, Timofeeff-Ressovsky 1932) We attain greater generality if we conceive any gene A as potentially existing in a number of alleles, say, A_1, A_2, A_3, A_4, A_5, etc., where, further, we may take the subscripts as indicating the level of activity of the allele Then in a certain population we may have a number of different A alleles, with perhaps A_2 most frequent, but a number of A_1 and A_3 and a few A_4 and higher values A genetic disturbance such as artificial selection will bring about a shift in the frequency distribution of alleles, A_4 may become the modal type, accompanied by fair numbers of A_3 and A_5 There might be no change in the level of heterozygosity at all

The simplest form of normalising selection would occur if, when such a disturbed population was again left to the action of

natural selection, the frequency distribution of the *A* alleles shifted back to what it had been before This would be a true genetic homeostasis But it would be optimistic to suppose that Mother Nature is always so simple *It is probable that the A* alleles are not the only genes contributing to the particular aspect of the phenotype which is in question There may be a *B* locus, with alleles B_1, B_2, B_3, B_4, B_5, etc , which for simplicity we may suppose to act additively with the *A* alleles Under natural selection, the distribution of *B* alleles will clearly have to be appropriate for the distribution of *A* alleles, and vice versa. Any artificial selection will probably shift both distributions, but if there are several such loci, and particularly if the relations between them are not simply additive, the distributions of the various sets of alleles in a population may be in unstable equilibrium, so that *after genetic disturbance the original phenotype may be restored* by a return to an allele-distribution which is not the same as the original one That something of this kind may actually occur is suggested by the results of Dobzhansky and Pavlovsky (1953) and by the variation in the results of identical solution experiments described by Clayton, Robertson and others (1957, see Fig 16, p 119) The mechanism which has been suggested is essentially similar to that of the 'drift reinforcing processes' discussed later

In both the processes described in the last paragraph a genetically disturbed population is brought back to phenotypic normality by changes which alter the gene frequencies but do not make any alteration to the degree of canalisation of the developmental system Thus both processes fall under the general heading of 'normalising selection', but they are two different varieties of it We may perhaps call a process which restores exactly the original genetical situation a 'homeostatic selection, for one which brings into being a genetic situation which is phenotypically equivalent to the original, but actually different from it *in the frequency distribution of alleles*, one can employ the phrase 'quasi-homeostatic selection'

The escape from selection

Natural selection is a stochastic process When, in the mathematical theory, a selective coefficient is attached to a certain allele

or genotype, this indicates the relative number of offspring which would be left, on the average, by a large number of individuals of that type forming part of an infinite population. In practice, populations are not infinite, and may be so small that it is not

[illegible]

pure chance rather than those of natural selection Sewall Wright (1931, 1932) and probably the majority of geneticists following him, believes that in Nature many effectively isolated populations may be of such a size that this factor of chance can play an important role, together with natural selection, mutation, migration, etc, in determining the directions of evolutionary change If this were so, the great importance of the phenomenon would be, not necessarily that such processes occur very commonly, but rather than when they do occur they would produce a considerable change which is unrelated to normal selective pressures The actuality of this so-called 'Sewall Wright effect', or 'drift', has

[illegible]

effects will soon be obliterated as the population once again becomes numerous enough to play a part in general evolutionary progress

The question is of great theoretical importance One of the most striking characteristics of modern evolutionary theory and one which has given rise to much philosophical controversy is the great importance which it attributes to random unforeseeable chance In the standard neo-Darwinian formulations of the theory all new variation is supposed to originate by chance mutation of genes This is a conception which requires some further discussion (see p 189), but in so far as we accept it, we have to suppose that the raw materials of evolution appear 'at random' without rhyme or reason If natural selection is the sole agency which controls their survival, some degree of orderliness is brought into the general picture of evolutionary processes, since of the random products of mutation only those will persist which enable the

organism to meet more efficiently the demands of the environment in which it lives If deviations from these stochastic laws of natural selection occur to any large extent in Nature, this orderliness becomes diluted Under these circumstances the frequencies of the various genes in a population would not be related strictly to their effect on the fitness of the animals bearing them, but would, as it is said, 'drift' in an unpredictable manner which was not related to anything in particular

From the theoretical point of view there seems no doubt that such a process of drift is a possibility The magnitude of the effect would depend on certain relations between the effective number of breeding individuals in a population, the intensity of the selective forces acting on the various genes, the amount of migration, mutation and so on. Unfortunately the mathematical theory suffers from the drawbacks which affect nearly the whole of the elaborate body of evolutionary theorising in mathematical terms In the first place, it is only possible to give an explicit algebraic formulation of the situation if one makes certain assumptions which, in fact, one knows to be almost certainly unjustified For instance, one must assume that the selective coefficient of any particular gene *A* remains the same whatever its frequency in the population and whatever other genes it is combined with. Sewall Wright, who is responsible for much of the theoretical work in this field, is well aware of this and has, in fact, provided several discussions of the matter (e g 1932) which are couched in broader terms and take account of the necessity to argue in terms of complete genotypes rather than individual alleles However, in order to do this one has to be content with expressing the various possibilities in a qualitative manner Wright, in fact, describes them in terms of a multi-dimensional phase space of gene frequencies, similar in its general nature to the phase space which we used earlier (Chapter 2) in discussing developmental processes Such a mode of expression can be very valuable in providing a set of terms—or even a visual model—in which one can work out ideas which are otherwise difficult to formulate In connection with the particular problem of drift, however, it loses touch with the point which is of crucial importance, namely

the quantitative The question at issue is not whether drift can occur but whether it would do so with values of population size, selective pressure, migration, etc , which it is reasonable to assume might occur in Nature Even if we are content with the simplest formulations of the mathematical theory which yield explicit algebraic expressions containing these parameters, we can in practice hardly apply them, since the quantities concerned are exceedingly difficult to measure under natural conditions, while if we proceed to a more profound and convincing theoretical account of the situation, even any explicit reference to the parameters disappears and we find ourselves left with nothing which will help us to decide how important the process of drift will be in Nature

We have, then, to abandon the theory except as a qualitative expression of the possibilities and turn to an examination of the actual phenomena in Nature to try to discover how far drift may have played a part in their causation. Certainly it seems at first sight that many of the results of evolution are of a kind which could most easily be explained by a process of random change in gene frequencies Many, if not most, nearly-related species differ in characters the selective value of which is not obvious It is, of course, always possible that minor differences in, say the distribution of bristles on the body of a fly, or in the proportions of some appendage of the body, have some unsuspected importance in the animal's life and that differences in these respects have been brought about strictly by natural selection Indeed, there have recently been several examples in which such apparently trivial differences have actually been shown to be more important than they looked (e g Cain and Sheppard (1950) on colour differences in the shells of the snail *Cepea*) Nevertheless, it is by no means obvious that one is justified in relying on such hypothetical selective advantages to explain all such cases

In particular, it is rather common to find that just outside the edge of the main area of distribution of a species there are small more or less isolated pockets of it, perhaps inhabiting actual islands or in some other way cut off from the main bulk of the population, and that these isolated groups each have their own

peculiar characteristics The greater degree of divergence shown by such 'island' populations as compared with the main large, continuous population of the species, has been considered by most authors (e.g Huxley 1942, Mayr 1954) to provide strong evidence for the reality of drift processes in Nature However, not all students of the subject agree on this For instance, Dowdeswell and Ford (1953) point out that the divergence of island populations may result from the fact that each such isolated group becomes adapted by natural selection to the particular demands of its individual habitat without the danger of such adaptations being swamped in the early stages of their evolution by inter-crossing with the main population which must be adapted to the average conditons of the territory it inhabits It seems plausible enough that the factor to which they draw attention may have played an important role in many cases However, it may be acting as a supplement to drift rather than as an alternative

These authors also draw an important distinction between two types of process, both of which would probably usually be included under the heading of drift, although they only refer to the first of them in this way They use the term 'genetic drift' of a

ence on the frequencies of the various genes, and they class as a separate phenomenon an occasional drastic reduction in the numbers lasting only a generation or two The former of these one might call 'persistent' drift In my opinion the second item merits the name of intermittent drift Dowdeswell and Ford argue that in the cases they have studied the populations are normally too large for it to be plausible to suggest that persistent drift is occurring On the other hand it is of course, very difficult to exclude the possibility of intermittent drift

The situation which Dowdeswell and Ford studied was the frequency of various types of spotting on the wings of the butterfly *Maniola jurtina* on various islands on the Scillies group The populations on three larger islands were very similar but those on a number of small islands rather diverse On none of the islands

at the time they were studied were the populations small enough for persistent drift to be likely to occur, but the possibility is admitted that the small islands may have been initially colonised by very small numbers of individuals or at some time in the past a natural catastrophe or epidemic may have reduced the population to an extremely small size so that a process of intermittent drift would have happened Ford himself has sided with Fisher (Fisher and Ford 1947, 1950, etc) in a rather bitter controversy against Sewall Wright and the theory of drift, but Dowdeswell and Ford's own data seem to find much their simplest explanation in the occurrence of intermittent drift, and they certainly provide no compelling ground for rejecting the possibility

The investigation of actual situations as they confront us in Nature does not therefore provide adequate grounds for denying the possibility that drift, particularly of the intermittent type, may sometimes occur There are further theoretical grounds which have not yet been discussed which suggest that any tendency for the gene frequencies in a population to escape from the control of natural selection, even temporarily, might have more important consequences than would appear at first sight There are, indeed, good reasons to believe that the natural structure of a population will provide something which might be described as a 'drift-reinforcing mechanism' The selective value of any particular gene in a population will depend, to some extent at least, on the other genes which are present with it and with which it may be combined If, owing to a stochastic aberration, only a statistically incomplete sample of the population survives to breed, the selective values of the surviving genes may have been materially altered by the change in the range of partners which is all that the population now offers them In so far as this occurs, natural selection will tend to pull the gene frequencies in the new population towards an equilibrium different from that which characterised the earlier situation. It is difficult to deduce from any general grounds how widespread or important such phenomena may be Exploration of the situation, using artificial populations, is now proceeding in several laboratories One must, however, at least envisage the possibility that changes in gene frequencies

produced by the fluctuations of sampling may be in practice more or less irreversible and perhaps lead to ever increasing divergence between the old and the new populations (Mayr 1954)

We must, I think, conclude that present day views tend to suggest that evolution is an even more contingent and unpredictable process than earlier students had thought In the past most biologists even though they held views as different as those of Lamarck and Darwin, have stressed the dependence of the changes which occur in an animal's evolutionary history on the nature of the environmental demands made upon it If drift is a real phenomenon it will, to some extent, mitigate the stringency of the test which the environment applies It will remain true, of course, that an evolving group of animals will only survive if it can at least cope adequately with the conditions of its life, but it will no longer be true that the race goes always to the swiftest It may in some years be the pass-degree man, and not the senior wrangler, who gets the fellowship and stays on at the world's high table to pass on such qualities as he possesses to the next generations which try to pass Nature's examinations

The contribution of the environment

It is, of course, a truism which has long been recognised that the development of any individual is affected both by the hereditary determinants which come into the fertilised egg from the two parents and also by the nature of the environment in which the development takes place However, during the early history of the science of genetics the main endeavour was concentrated on discovering the laws of inheritance, that is to say the rules by which hereditary factors are passed on from one generation to the next The presence of these factors has to be recognised from the phenotypic appearance of the individuals concerned and any effect of the environment in modifying these appearances would be a disturbing element Geneticists therefore usually attempted to study variant forms whose appearance was not altered by the range of environments to which the experimental animals were subjected

There is a type of *Drosophila* which, under normal environments, has a noticeably small eye The effect is passed on from one

generation to the next and is due to a dominant gene, known as *bar*, on the sex chromosome Now if we have a series of individuals containing the *bar* gene and brought up at a number of different temperatures, the size of the eye (which can be estimated by counting the number of eye facets) is smaller in those which have grown at a high temperature than in those which have grown at a lower But even at the lowest temperature which will allow the flies to develop reasonably fast, the eyes of *bar* flies are not as large as those of normal individuals The presence of *bar* can therefore always be satisfactorily recognised and, in the context of an investigation of how it is passed from parent to offspring, one can neglect the effect of the environment on the expression of the character

In elementary expositions of the Mendelian laws of inheritance there is often little or no explicit reference to the part played by the environment in influencing development This rarely leads to any serious misunderstanding among biologists, since, as we shall see, very considerable discussion of the subject has gone on since the earliest days of genetics However, certain authors interested in the methodology of science have been deceived by the lack of reference to the environment in accounts of elementary Mendelism into thinking that geneticists have failed to realise its importance

Woodger argues that the relevance of the environment to hereditary problems should always be explicitly recognised, and he proposes that this should be done in the following way He begins by asking the question what do we mean by 'inborn'? If, for instance, we say 'Tom has red hair', what is involved if we assert that the red hair is inborn? He begins by pointing out that we certainly do not mean that red hair, as such, is derived directly from the parents since there is obviously no hair in the egg. Again, he points out that 'to say that redness is handed down from parent to offspring is clearly metaphorical' This is, of course, all quite true, and what we do in fact say is that the egg from which Tom developed received from the parents a *something (a hereditary factor* or, to use the recent word, a gene) which influences the developmental reactions in such a way that the hair which grows

on Tom's head will contain red pigment Obviously much more might, and indeed has, been said about the relation between the hereditary factors and characters, but it is for the most part quite *irrelevant* to the problem which Woodger had set out to discuss, namely the influence of the environment, since factors and characters would still belong to different categories even if the environment did not affect development at all

Woodger, therefore, drops this question and takes up the discussion of the environmental effects on development He considers a set *X* of fertilised eggs (zygotes) developing in a set *Y* of environments into a set *P* of phenotypes If the set *X* contains all the zygotes which may under some circumstances or other develop into phenotype *P*, and *Y* contains all the environments under which some zygote can develop into *P*, then he calls *X* the zygotic range of *P*, and *Y* the environmental range of *P* On this basis he defines his fundamental concept, that of the environmental insensitivity of *P* He says that *P* is environmentally insensitive, if every member of *X* always develops into *P*, provided it can develop at all On the other hand, if there are environments in which members of *X* can develop, but do not in that environment come to exhibit the phenotype *P*, then we can say that *P* is environmentally sensitive Woodger suggests 'that there would be fewer misunderstandings in biological science and fewer misleading statements in popular biology if people ceased to speak of inborn and acquired characters and spoke instead of environmentally insensitive and environmentally sensitive phenotypes'. Wisdom, in reviewing a book of Woodger's, in which this theory was put forward in a preliminary form, agrees with this suggestion, and feels that Woodger has 'taken a fundamental step in the clarification of the subject' It is somewhat disconcerting to the ordinary biologist, however, to discover that both Woodger and Wisdom seemed to think that one of the points on which they are for the first time admitting a little light is the belief that an inborn character is necessarily incurable, an elementary confusion which one had thought was nowadays forgotten

In point of fact, Woodger's concepts suffer from a number of deficiencies which render them rather unattractive in comparison

with those which geneticists commonly use in this context His is a logician's definition, set out in terms of concepts which are of an all-or-none character, and therefore intellectually clear-cut but in practice more or less inapplicable For instance, if we consider a particular phenotype, such as that of a human being with skin of a particular shade, it is in practice quite impossible to discover its zygotic range by finding all the zygotes which could, under some circumstances or other, develop that skin and colour. One could perhaps obviate this difficulty by comparatively slight modification of the definition More serious is the fact that the definition treats phenotypes as though they can in general be sharply distinguished from one another, so that an individual either belongs to phenotype or does not belong to it We have seen in the case of *bar* mentioned above that such distinctions can sometimes be made, but only if one allows a given phenotype (say that of *bar*) to cover a certain range of variation It is in practice more frequent, in the contexts in which this subject is important, to find that different environments affect the degree of expression of a particular phenotype, rather than that they decide whether the individual will belong to that phenotype or not belong to it.

Following Timofeeff-Ressovsky (1927), geneticists commonly use the word 'expressivity' to indicate the degree of expression of some phenotypic character, and 'penetrance' to indicate the proportion of a given set of zygotes which develops into adults which exhibit some recognisable phenotype Woodger's definition is essentially in terms of penetrance From this it is not easy to deduce anything about expressivity, which is the practically more important phenomenon Moreover, if we have a theory in terms of expressivity it is quite easy to modify this so that it can deal with penetrance, since we can always suppose that the proportion of a set of zygotes which develop a certain phenotype depends on the number among them in which some underlying continuously variable character is expressed to a degree greater than the necessary threshold

Let us consider then what geneticists have actually done to try to handle the questions raised by the influence of the environment on the phenotype, and first it will be as well to glance at the type

of phenomenon with which they are confronted I take an example which dated from many years ago, and which might well have been in the minds of Woodger and Wisdom when they discussed the matter The data come from the work of Krafka (1920) and I quote the discussion of them given by Hogben (1933)

'In the fruit fly *Drosophila* there is a series of mutations characterised by extensive reduction of the number of facets in the compound eye Two such mutations are designated "low-bar" and "ultra-bar" The actual number of facets varies with the temperature of the environment in which the larvae develop In the diagram (Fig 11), the distance *AB*, measured along the ordinate *ABC*, represents the difference ($_{16}\delta_{H}$) of the two stocks, both cultured at 16° C The distance *EF* represents the difference between the two stocks, both cultured at 25° C The length *BC* represents the difference between the measurement of ultra-bar individuals cultured respectively at 16° and 25° C The length *DE* represents the difference between the facet number of low-bar individuals cultured at 16° and 25° C respectively *EF* and *AB* each correspond to what the experimental biologist means by a genetic difference *BC* and *DE* each represent what the experimental biologist calls a difference due to environment

Clearly we are on safe ground when we speak of a genetic difference between two groups measured in one and the same environment or in speaking of a difference due to environment when identical stocks are measured under different conditions of development Are we on equally safe ground when we speak of the contribution of heredity and environment to the measurements of genetically different individuals or groups measured in different kinds of environment? Suppose we measure a low-bar stock kept continuously at 16° C and an ultra-bar stock kept continuously at 25° C The observed difference will be represented by the length *AC* or *DF* How much of *AC* or *DF* is due to heredity and how much to environment? The question is easily seen to be devoid of a definite meaning We might be tempted to say that the genetic contribution is the difference which would exist if both stocks had been cultured at the same temperature This could be done in an infinite number of ways If they were

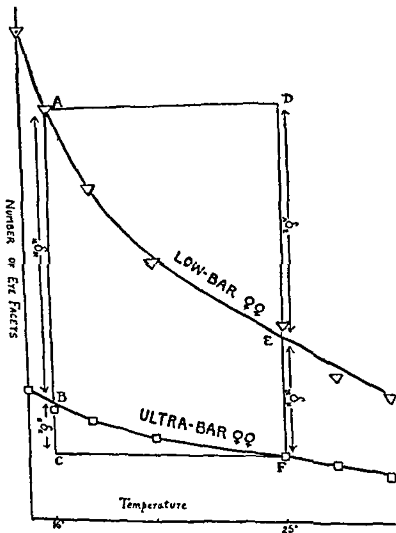


FIGURE 11
The effects of temperature on the number of eye-facets in the two stocks low-Bar and ultra-Bar (From Hogben 1933)

both cultured at 25° C, heredity would contribute *EF* If they were both cultured at 16° C, heredity would contribute the difference *AB* We might also be tempted to say that the contribution of environment represents what the difference would be if all the flies belonged to the same stock. Obviously this can be done in at least two ways Keeping the same difference of environment we might substitute low-bar individuals for the ultra-bar stock at 25° C. The difference between the two sections of the population would then be represented by *DE* If we substituted ultra-bar individuals for the low-bar stock at 16° C, the difference would be *BC* Either *ED* or *BC* is equally entitled to be regarded as the contribution of environment'

In discussing this example in modern terminology we should tend to use the expression 'environmentally sensitive' of the developmental reactions controlled by the genotypes rather than, as Woodger does, of the final adult phenotypes Thus we should say that the low-bar genotype causes the epigenetic system to be environmentally sensitive, meaning that the kind of phenotype (in particular, the degree of expression of the phenotype) finally exhibited by a zygote of low-bar genotype would depend on the environment in which it developed Similarly, the development of the ultra-bar genotype is environmentally sensitive but to a different degree Such a difference of degree in environmental sensitivity of the development controlled by two genotypes is spoken of as 'genotype-environment interaction' This expression is derived from statistical terminology and, as this example makes clear, is used in a much more restricted sense than might appear at first sight It is to be avoided when one wishes merely to indicate that the phenotypic effects of a genotype are influenced by

Geneticists have been attempting since the very early days of their subject to formulate some method of handling the simultaneous contributions of the genotype and the environment to the phenotype They have approached it through the consideration of cases in which the phenotype varies not in an all-or-none

but in a continuous manner (More recently a treatment of all-or-none phenotypes has been derived from that originally worked out in relation to quantitative variation, see Wright 1943, Lush, Lamoreux and Hazel 1948, Robertson and Lerner 1949) Moreover, they began with the simplest kind of quantitative variation, namely that which takes place in one dimension so that the grade of expression of the phenotype can be expressed on a single scale Their procedure was to consider a set of genotypes X which develop in a set of environments Y into a set of phenotypes Z, and they then attempted to assess the contributions of the genotypes and of the environments to the variation which is found in the phenotypes

The first analysis of variability into genetic and environmental contributions which took account of the Mendelian discoveries was given as long ago as 1909 and 1910 by Weinberg For a recent account of the theory which follows essentially the same method of approach one may take the formulation of Lerner 1950 Taking the total variance of the phenotypes as σ_P^2 he writes,

$$\sigma_P^2 = \sigma_{Ge}^2 + \sigma_E^2 + 2r_{GeE}\sigma_{Ge}\sigma_E + f(GeE)$$

where Ge stands for the genotype E for the environment, and r_{GeE} represents the correlation between genotypic and environmental variations and the term $f(GeE)$ stands for non-linear ge[illegible]

ph[illegible]

still too complex to be made much use of as it stands It is considerably easier to handle if one is justified in omitting the last two terms on the right, and in most attempts to apply it, this simplification has been tried in the hopes that practice would show that it was not too far from the mark. If this is done, the fraction of the total variance in phenotypes, which is directly due to genetic differences becomes σ_{Ge}^2/σ_P^2 This fraction is known as 'heritability', in the broad sense of the word Far from geneticists requiring to be reminded that the environment plays a part in influencing phenotypes, the concept of heritability, which obviously makes allowance for just this fact has become a focal

notion in most recent genetical treatments of the relation between genotypes and phenotypes, particularly in connection with the breeding of agricultural livestock.

There are, however, considerable difficulties in making use of the concept of heritability in practice In order to estimate its value we require to measure σ_P^2 and σ_{Ge}^2 It is relatively easy to assess the former, if the phenotypes vary in a uni-dimensional manner, since we can then measure the degree of expression of each phenotype on some scale and compute the total variance of the set of individuals we are dealing with. We cannot, however, directly measure σ_{Ge}^2 If we know the degree of relationship between the set of genotypes X with which we are dealing (for instance, if they are all full brothers and sisters or are a group of 'half-sibs' with one father and a large number of mothers) we can attempt to assign a value to σ_{Ge}^2 from general considerations as to the nature of Mendelian inheritance This can, however, usually only be done if we make certain assumptions as to the nature of the reactions between the various genes in the genotype. The simplest such assumption is that the genotype contains numerous genes affecting the phenotype in question, each gene adding a fixed amount (positive or negative) to the measure of phenotypic expression In Lerner's terminology we write σ_{G}^2 for the variance due to additive action of this kind, and it is common practice to use this in place of σ_{Ge}^2 in the calculation of heritability When this is done, that concept is being used in a somewhat restricted sense, but in practice little is lost, since the contribution due to σ_{G}^2 is the only one of importance in controlled animal breeding Heritability in this sense is usually referred to as h^2 This coefficient is of considerable practical importance, since if one is attempting to improve the genetic constitution of a population of animals by a programme of selective breeding, it is the h^2 which determines how rapidly progress can be made One can, in fact, obtain a measure of h^2 by finding the ratio between the amounts by which a group of selected parents on one hand and their offspring on the other exceed the average phenotype of the population from which they were drawn.

The whole subject of utilising the concept of heritability is a

highly complex one which has a considerable literature of its own (for a recent discussion of it see Lerner 1950) I do not wish to enter into the details of these matters here, since their main importance lies in the field of animal breeding, in which quantitative estimates of the rate of response to selection, of the results

In the first place it must be remembered that any estimate of heritability applies only to one particular set of genotypes in one particular set of environments If we have another set of genotypes with different genotypic variability, or another set of environments, the value of the heritability coefficient will be altered Even when dealing with one set of genotypes in one set of environ-

calculated from the response to selection Falconer (1953) suggests that the latter be known by the qualified term of the 'realised heritability' The differences between the heritability and the realised heritability are due partly to the simplifying assumptions involved in neglecting the correlation between genotypic and environmental variance and the genotype environment interactions, partly to the other simplification of substituting σ_G^2 for σ_{Ge}^2, partly to effects produced by the frequencies with which various genes are present in the population, and in many cases also to the occurrence of natural selection simultaneously with the artificial selection which is being practised There is a great deal of scope for logical analytical thought in clarifying this situation, and it is in this connection rather in the simple point that the environment has some influence on the phenotypes that geneticists would be grateful for assistance from those who have training in methods of logical analysis

The fullest experimental study of the applicability of heritability theory is that which has recently been made by Clayton, Robertson and others (1957) They point out that the theoretical

predictions of the results of selection can only be expected to be accurate so long as one may assume that the 'genetic parameters' of the population remain unaltered While this is the case, the predictions of the theory are not dependent on the exact nature of the dominance or epistatic relationships of the genes, and thus the existence of developmental canalisation would have little effect on the response to selection In the material they studied, namely the abdominal bristles of *Drosophila*, there is good evidence that the character is of little direct importance for natural selection (see p 118), so that in any case it would not be expected to show much evidence of canalisation And in practice they found that the theory gave reasonably accurate prediction of the selection effects over the first few generations

Even for the early generations of selection, the heritability theory can predict only the phenotypic response to selection It is impossible to specify the extent to which gene-frequencies will be altered, unless one makes assumptions as to the types of gene action and interaction which are occurring in the population, that is, assumptions about such matters as dominance, epistasy and other aspects of canalisation Clayton and Robertson found that in later generations the theory became of no predictive value, since the genetic parameters had changed considerably in consequence of alterations in gene frequencies whose nature and effects there was no way of foretelling We badly need new methods of studying the genetic set-up in a population in such a way as to give the information essential for estimating how the epigenetic situation is likely to alter as the gene frequencies change It is difficult to see how this problem could be profitably approached except through developmental or physiological analysis How else could one expect to find means of foretelling when, for example, a favoured gene is likely to start behaving as a recessive lethal, as so many did towards the end of Clayton and Robertson's experiments? An example of one type of physiological investigation of a problem of this kind, involving testing the effect on the population of a variety of different environments, will be found in Dobzhansky, Pavlovsky, Spassky and Spassky (1956)

These problems which arise within the heritability theory are,

perhaps no more than might be expected in any subject which is rapidly growing and advancing And fortunately they do little to reduce the value of the theory in the field in which it has most practical application namely in connection with the breeding of livestock, where one is hardly concerned with more than a sequence of very few generations But in the context of more general biological problems the major defect of the theory is the narrowness of the field which it takes into account The study of heritability has in the main been carried out from two points of view Firstly by people interested in the correlation between the phenotypes of related persons in human populations, and secondly, by those concerned with animal breeding Both groups of investigators have tended to concentrate on the study of some one particular phenotypic character such as height, weight, milk yield etc and they have relied on statistical methods for analysing it The subject has fallen victim to the two dangers which statistical methods always seem to bring in their train.

In the first place there has been a tendency to regard to a refined statistical analysis of incomplete experiments as obviating the necessity to carry the experiments further and to design them in a more penetrating fashion For instance, if one takes some particular phenotypic character such as body weight or milk yield one of the first steps in an analysis of its genetic basis should be to try to break down the underlying physiological system into a number of more or less independent factors Are some genes affecting the milk yield by increasing the quantity of secreting tissue others by affecting the efficiency of secretion, and others in still other ways? I have recently discussed a case of variation in the quantity of vein formed in a certain region of a *Drosophila* wing in which it was particularly clear that the genetic systems fell into distinct physiological groups in this way (Waddington 1955) This was made obvious owing to the fact that visual inspection of the wing reveals not only the quantity of the venation but also the pattern in which it is arranged A normal quantity of venation may be present either as a single continuous vein or as a shortened vein to which a piece is added at the side Another example will be found in a study by Forbes Robertson (1957) on

the inheritance of adult size in *Drosophila*, in which attention is paid to the different contributions of increase in cell size and in cell number With most phenotypic characters a physiological analysis of the basis of the variation, even of such a relatively superficial kind as in these examples, cannot be made so easily It cannot even be begun by any form of arithmetical manipulation of measurements of the global total of the character as a whole It demands specially planned experiments aimed at isolating the different variables concerned Such investigations have, however, been very generally neglected

Secondly, the statistical techniques at present available, although imposing and indeed intimidating to most biologists are in fact very weak and unhandy tools The first partition of variation into a genetic and an environmental component by Weinberg (1909 1910) was based on very drastic simplifying assumptions as to the kind of genetic system involved Fisher (1918) made a major advance in giving a mathematical treatment of considerably greater generality and further advances have since been made by Wright (1952), Malecot (1948) and Kempthorne (1954) But after nearly half a century's development the statistical theory still has to leave out of account the contribution of genotype-environment interactions represented by the last term on the right in the equation on p 95 These interactions are that part of the variation which rises from particular genotypes being specially affected by particular environments Now from the point of view of the theory of evolution such special interactions between genotypes and enviroments are obviously by no means negligible In fact the whole of adaptive radiation, including the formation of local races turns on the way in which particular genotypes fit into certain environments that is to say, on this very factor of genotype-environment interaction

From the point of view of evolutionary theory, the most important defect of the current statistical method of dealing with the relation between genotype and environment is that it concerns itself *only* with uni-dimensional variation That is to say, it takes some particular phenotypic character which can be measured on some single scale and discusses how alteration of the environment

induces changes in the measure of the character on that scale Now in point of fact any given alteration in the environment will normally affect a developing animal in many different ways A colder climate may produce not only a change in total body size but also perhaps alterations in the proportions of the limbs and body, in the thickness of the coat, in the amount of food consumed, and possibly in many other characters Any of these environmentally-produced changes may play a part in the evolution of the population in which they occur but there is no *a priori* method of deciding which of them will become important in this way A treatment which arbitrarily chooses some one character to measure and which confines itself to trying to discover how much of the variation in that character in a given population is due to the environment, can at best only be wise after the event, when the fullness of time has revealed which of all the environmentally-produced modifications is the important one But it obscures the fact that any one environmental stimulus causes many different kinds of variations Thus it provides a quite inadequate basis for discussing the evolutionary importance of environmental change

It issues, in fact, in the notion that natural selection is concerned solely with that component of variation which is handed on directly from parent to offspring, and that the environmental component of variation is nothing more than an unfortunate complication which prevents natural selection working as efficiently as it might The genetic system can be regarded as a mechanism for transmitting, from the parent generation, a quantity of 'information' which can serve to determine the character of the offspring The implication of the methods at present available for the statistical analysis of variation is that the environment acts on the system merely as 'genetic noise' which reduces the efficiency with which the information is conveyed This is undoubtedly an over-simplification When a mixed population suffers an alteration in the environment the development of some partic lar genotype [illegible]

of genotype and environment that evolutionary modification of the population takes place

In current evolutionary theory, this debasement of the role of the environment takes the form of an attempt to frame the discussion as though selection acted directly on the genotype It is true of course, that selection only has effect on later generations in so far as it controls the genes which are *transmitted It has, therefore,* some meaning to attach to each gene a coefficient expressing its net effect on fitness, 'taking account of all the genotypes into which it enters', as Sewall Wright puts it (1956) And one can add to this picture the consideration that when the environment alters there is, in his words, a 'shifting of the goal toward which mass selection is directed', that is, the selection coefficients change But although this *procedure is meaningful up to a point* and has in fact led to very great advances in our understanding of evolutionary processes, it is not meaningful enough, it does not take cognisance of all the important elements in the situation For one must ask the question, why does a certain gene have a given selection coefficient? The answer must be in the form that the gene has a particular effect on the developing organism, and the *resulting phenotype has a certain fitness, and this inescapably leads* to the consideration that the phenotype is a joint product of genotype and environment together

This point is, of course, not at all a novel one, and is well recognised by mathematical geneticists themselves For instance, Crow (1955) begins a recent summary article with the words 'A full quantitative theory of evolution would be impossibly complex For example, it would have to consider adaptability as well as adaptedness, for in the long view the former must also be important Such a complete description is far beyond the capacity of workable mathematical models ' The reason why so much stress is being laid on the point here is in order to overcome the anaesthetic effect exerted on *biologists by algebra which* is incomprehensible to them There is no need for evolutionary theory to limit its view to those topics which have been successfully formulated in mathematical terms Nor need one accept Crow's suggestion that the mathematically difficult concepts,

such as adaptability, are important only 'in the long view', some of them look like being the crux of the matter here and now. It is perhaps, only if the biologist continues to do his poor best to cope with such problems with the imprecise but subtle tools of ordinary language that the mathematicians will eventually be driven to dissect them with their penetrating but relatively inflexible scalpels

The comparison between the two points of view can perhaps be symbolised by the diagrams in Fig 12 In the upper, which

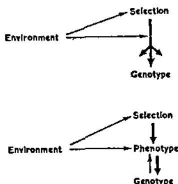


FIGURE 12

The relation between the environment and natural selection In the upper diagram (the conventional view) the environment influences the selective [illegible] mitted to it In the lower diagram, selection, again influenced by the environment, acts directly on phenotypes which are determined both by the genotype and the environment it acts on genotypes only secondarily

shows the conventional view, the environment determines the selective forces, and they act directly on the genotype, if, in such a formulation, any attention at all is paid to the effect of the environment on phenotypic variance, this can be represented as introducing an element of 'noise', or arbitrary imprecision into the relation between selection and genotype In the second diagram, which represents in very simplified form the point of view

being advanced here, the environment not only determines the selective forces, but also co-operates with the genotype in the specification of the phenotype, and it is on the latter that selection acts, its influence on the genotype being secondary

The experimental biologist will, perhaps, not be very interested in discussing which of these points of view is logically the better based The best justification for introducing a new point of view is that it enables one to advance some new hypothesis which can be experimentally tested When we emphasise that selection operates on phenotypes which are the joint resultant of genotype and environment, it is in the context of adaptation that we naturally find ourselves, since we are in fact suggesting that all natural selection is in fact a selection for the ability of the organism to adapt itself to (that is, to develop into a fit form in) the environment in which it finds itself In later chapters, some definite hypotheses will be drawn for such considerations, and some experiments bearing on them reported But before we leave the general subject of the role of the environment in evolutionary processes, there is another aspect of it to be remembered

Selection of the environment by the animal

One can have at best a very partial view of the processes of natural selection if one leaves out of account the fact that not only do environmental conditions exert selective forces on the animals but that there is a complementary selection by the animals of the particular circumstances to which they will subject themselves The ambience (p 132) surrounding an animal—that is the range of environments open to it—is probably always to some extent heterogeneous either in physical conditions or in possible relationships with other inhabitants of the locality Within this gamut of circumstances the animal has some freedom of choice It is of course, a platitude of natural history that nearly related species, presumably derived from one ancestral population, are now found to occupy ecological niches which are different either in their geographical location or in some other way Elementary theory would suggest that within any widespread population there must be a genetic diversity in habitat preferences which could serve as

a basis from which such specific divergence might be built up The subject has, however, been rather neglected in recent years and receives only the most cursory notice in books such as Huxley, while even those who pay somewhat more attention to it (e g Cushing 1944, Thorpe 1945, Mayr 1951) tend to give the impression that it is only a side issue in a general structure of evolutionary theory. As Crow and Kimura (1956) have pointed out, evolutionary mathematics have passed through two major phases The first dealt with deterministic processes, in which the environment of the evolving population was considered as uniform, in the second, stochastic processes were taken into account, first in relation to random fluctuations in gene sampling, and later, at the hands of Kimura himself, in connection with fluctuations of the environment It seems clear that there is a third phase still to come, in which the circular relation between the animal and its environment will be expressed in terms of the mathematical concepts of cybernetics or of the Theory of Games

A good example of a genetic difference in habitat preference existing in a wild population in Nature has been described by Hovanitz (1953) The butterfly *Colias eurypheme* occurs in California in a white form which appears only in the females and is dominant, and in various shades of yellow and orange Hovanitz found that the percentage of white forms active in an alfalfa field was much higher in the early morning and late evening than in the middle of the day It is clear that one of the effects of the white genotype is to determine in the animal a more marked preference for the environmental characteristics of those periods—a combination of light and heat are probably the two most important factors Again Dobzhansky and da Cunha (1955) have used different strains of yeast to attract and trap the *Drosophilids* of the Brazilian fauna They found, not unexpectedly, that there are many species which showed marked differences in their responses to each of the four yeasts tested There were five species which occurred in considerable frequency in several of the different areas of Brazil in which the trapping experiments were carried out Of these, four showed some evidence that the food preferences are not constant throughout the distribution area of the species It is

not perfectly clear, however, how far the preferences shown by a given local race depend on what other competing species of *Drosophila* occur in the same region Nor, from the nature of the experiment they did, could there be any evidence of the existence of genetic diversity in food preferences within a single population of one species from a particular locality However, laboratory experiments have revealed the existence of habitat preferences between genetic strains of *Drosophila* which have not undergone any evolutionary processing in the field These were demonstrated by the following experiment.

A box was constructed in which a central compartment communicated, by small holes, with eight more peripheral chambers In each of these the environmental factors of light versus dark, heat versus cold, damp versus dry, could be individually varied A mixed population of adult *Drosophila* were placed in the central compartment and left for some hours, after which the numbers which had moved into the various peripheral compartments were counted The mixed population was made up from a number of laboratory strains, each marked by a well characterised mutant Presumably these strains differed from one another in many minor genes as well as in the major marker genes It was found that not only were there considerable differences in the general activity of the various strains, that is, in the proportion which had moved out of the central compartment into one or other of the peripheral ones but that there were highly significant differences in the ways in which the various strains had sorted themselves among the available environments It was clear that the flies exhibited preferences for one or other environmental factor and that these preferences were not the same in all the strains (Waddington, Woolf and Perry 1954) Janzer and Ludwig (1953) have described rather similar evidence from an experiment in which different stocks of flies were released in a box one metre long, one end of which was illuminated while the other was kept dark. They found characteristic differences in the positions taken up by flies of different stocks five minutes after the beginning of the experiment.

If the existence of genetic diversity in habitat preferences in

wild populations is accepted, it is clear that we are confronted in evolution with a situation which is more complex than that usually considered (Fig. 13). It does not follow, of course, that



FIGURE 13
The four critical points (marked by black circles) *in the relation between a genotype and the forces of natural selection* (From Waddington 1954*b*)

this added complexity introduces anything of [illegible]
for our understandi[illegible]
hardly do so except [illegible]
the preferences which a given genetype shows for various environments open to it are correlated with its fitness in those different environments. One's confidence in the general effectiveness of natural selection is such that I think that one would expect

that most genotypes, if they exhibit any choice among the varieties of the environment available to them, would choose that in which their fitness is highest On a broad enough scale, this is certainly true, terrestrial species inhabiting regions which offer many varieties of habitat normally choose that to which they are adapted There is less evidence about the behaviour of intraspecific varieties, but it seems usual to find that cryptically coloured individuals tend to place themselves against the appropriate background, for instance, Kettlewell (1955) has shown that the melanic or pale forms of the Pepper Moth prefer to settle on the darker or lighter parts, respectively, of the lichened trunks of birch trees.

Habitat preferences which are correlated with variations in fitness might obviously play an important part in determining the course of natural selection The mere existence of a range of ecological niches, in each of which the selection coefficients have characteristic values, may sometimes suffice to preserve genetic heterogeneity in a population, in the absence of either habitat preference or assortative mating as Levene (1953) and Li (1955) have demonstrated. Under those circumstances, a stable polymorphism seems to be possible only if, for each gene, both the homozygotes and the heterozygote have different selective coefficients, moreover there have to be certain particular relations between the fitnesses of the various genotypes concerned If individuals of different genotypes exhibit preferences for those ecological niches in which their fitness is highest, this would certainly seem to increase the likelihood that there would be some condition of stable equilibrium to which the population could attain Another factor which would seem to tend in the same direction would be any tendency for the inhabitants of a given niche to mate with one another rather than at random with the population as a whole The theory of the situation, however, has not been fully explored mathematically.

CHAPTER 4

THE ORGANISATION OF THE GENE POOL

1 *Fitness*

CIVILISED MAN is, at least in theory, used to the fact that each individual human being is not entirely like any other This fact of individual diversity, on which so many of our most cherished political and religious doctrines are founded, is true also of organisms which we consider more lowly than ourselves Any single animal taken from a freely interbreeding natural population will be found to differ genetically, to a greater or lesser extent, from its fellows Its genotype contains a sample from the highly diversified collection of genes which is contained in the population as a whole, and which is known as its gene pool Recent authors, particularly Dobzhansky and his associates, have shown that the gene pool of a population is not a mere haphazard assemblage of genes which have no essential connection with one another save the bare fact that they occur in members of the same interbreeding group On the contrary, it seems that they are to some extent 'co-adapted', the presence of one gene having an influence on the chance that another will survive as a permanent member of the population's genetic resources Much of modern evolution theory turns on the analysis of this organisation of the gene pool and of how it arises and is maintained

This organisation is, of course, built up by selection, which operates on the fitness of the individuals in the population For the purposes of a purely formal theory, fitness can be given a simple definition, as the capacity to contribute offspring to the next generation Thoday (1953) has suggested that it should be defined with reference to a longer lapse of time, 'the fitness of a unit (of evolution),' he writes, 'is its probability of leaving descendants after a given long lapse of time.' This is, as he shows, an interesting concept to discuss, but it is not what is usually meant

by fitness in evolutionary theory, and it cannot replace the more conventional notion which is concerned only with the next generation. It is the latter which determines what evolutionary changes will happen here and now. Thoday's concept involves a notion similar to action at a distance, it is not a causal concept, since the Thodayan fitness of an individual existing now would be altered if some unforeseen or unforeseeable climatic change occurs even many years after it is dead

The normal concept of fitness, though easy enough to define for purposes of the formal theory concerned with changes in gene frequency, is by no means so simple when one tries to consider, in more concrete terms, how it is related to the phenotypic characters of organisms One may note in the first place that fitness, as it enters into evolutionary theory, is essentially a relative notion We are always concerned to compare the fitness of organisms of one kind with that of individuals of a different sort Further, fitness is a quality of the organism as a whole

It may be broken down, in a first analysis, into a number of immediate components of fitness, of which, to take *Drosophila* as an example, the length of fertile adult life, the mating ability, the rate of egg-laying, the hatching rate of the eggs, the percentage of larval survival, and so on, might be instances Each of these components in turn will be influenced by many characteristics of the organism, both morphological and physiological In no case has it yet been possible to complete the analysis of the fitness of an animal, and even to enumerate all the characters which enter into it, let alone to specify their relative contributions

Instead of starting with the notion of overall fitness and attempting to analyse it into contributory characters, one can begin from the other end, taking a character (such as eye colour, or wing shape, or number of bristles in *Drosophila*, or weight or thickness of fur in a mammal) and considering how variations in the rate or extent of its development, may affect fitness Such an approach is to some extent artificial, since it is always complexes of characters, rather than single isolated ones, which determine fitness Thus if we are dealing with characters *P*, *Q*, *R*, and *S* it might be that an efficient animal is produced if *P* has the value 1, *Q* 3, *R* 2

and S 4, but we must be prepared to find that equal efficiency might occur with some other constellation, for instance $P=2$, $Q=4$, $R=1$ and $S=5$

The problem of alternative genetic systems which lead to equivalent phenotypic fitness is probably more important when we are considering, not natural selection for fitness, but artificial selection for some more or less arbitrarily defined phenotypic character If one and the same phenotypic variant can be produced by different developmental mechanisms, each with its own genetic control, the selection will be operating on a character of the kind which has been referred to as 'genetically inhomogeneous' (Waddington 1955) For instance, the number of hairs on the abdominal sternites of *Drosophila*, on which so much selection work has been done, might be increased either by producing a higher density of hairs per unit area, or by enlarging the sternite leaving the density unchanged A f[illegible]

several plants, such as Primula, and for a very few animal examples, such as the guinea-pig coat colours analysed by Wright But unless it is possible to carry out the elaborate inbreeding and crossing necessary to obtain strains which differ at only one of the loci involved, and unless the allele-differences are sufficient to produce recognisable effects, the purely genetical techniques can hardly do more than indicate the general nature of the situation without being able to unravel it In such circumstances, little progress can be expected unless the developmental processes can be analysed into their component sub-systems by physiological investigations To give an example, Falconer and Latyschewski (1952) have studied the response to selection, for body weight at six weeks of age, of mice some of which were kept on a rich diet and others on a poorer one They were able to make some very interesting observations on the effects which a subsequent alteration of diet makes to the selected strains But each of the two characters selected for—weight on a good diet and weight on a poor diet—may have been causally inhomogeneous, on the good diet they may have chosen animals which had a good general

growth efficiency, as well as those which could flourish under soft conditions but not put up with hard times, while on the poor diet they would have favoured the first group but not the second If this were so, the genetic correlations which they calculated between the characters would have only a purely pragmatical significance

Sewall Wright, in particular, has emphasised the importance of such different adaptive facies He has expressed the situation in terms of a three-dimensional model, which in reality, like the 'epigenetic landscape', is a diagrammatic representation of a multi-dimensional phase space (Wright 1932) In Wright's model (Fig 14), the vertical dimension represents fitness, while the points on



FIGURE 14
Diagrammatic representation of the fitnesses of the field of genotypes which could be made up by varying the frequencies of various alleles in a gene-pool The fitnesses lie on a surface whose modelling is indicated by the contour lines (From Wright 1932)

the horizontal plane correspond to different combinations of gene frequencies in the population If there were only one 'best' composition of the gene pool, the model would consist of a single swelling surface, shaped like a hill the peak of which would correspond

with the fittest set of gene frequencies If there are several different fitness-maxima corresponding to different gene pools, there will be several hills, separated from one another by valleys which correspond to populations of lower fitness If the environment changes, so that the fitness of various phenotypes alters, the whole surface will be modified

The gene composition of any given population can be thought of as a point on such a surface Natural selection will normally act so as to drive the population up any slope on which it may lie, so that it will eventually arrive at a peak Once at a peak, the population will come to rest, i e its gene frequencies will settle down to an equilibrium, except for minor wanderings due to recurrent changes in the environmental circumstances or, if the population is small enough, to the operations of chance (p 82) The effects of such relatively small alterations in gene composition will depend on the configuration of the surface in the neighbourhood of the peak If the population occupies an isolated elevation which is surrounded by broad and deep valleys it may wander around the peak but cannot easily escape from it On the other hand, it may find itself at one of a number of peaks which differ only slightly in position and elevation, and it may then relatively easily pass from one to another

In the context of the evolution of natural populations such relatively minor degrees of genetical lack of stability are of little importance The environment of a population is usually to some extent fluctuating and this in itself will produce variations in the 'fitness-surface' of Wright's model The elevations will be more like the crest of an ocean roller in a choppy sea than a hilltop of solid rock, and the population will move about over a more or less extensive area There may, however, be broader and deeper valleys in the surface which might be of much greater significance Such features of the organisation of the gene pool are likely to be relat al

r

e

to another major elevated region

Goldschmidt (1940) has urged that there must be a difference in

kind between processes of 'micro-evolution', which lead to the appearance of groups of local races or nearly related species, and those of 'macro-evolution' which give rise to the larger categories Many species, he admits pass into one another by more or less insensible gradations, through local and ecological races, and these groups may well have become diversified by straightforward processes of selection, etc , of the kind usually considered in evolutionary theory But he argues that between these groups of related forms there are 'unbridgeable gaps', each set of related forms sharing some basic type of organisation in which it differs from all other groups He suggests that these unbridgeable gaps are evidence for the occurrence of a novel type of variation—a 'systemic mutation', which brings into being a completely new kind of genotype, much more profoundly different from the original type than could be produced by simple gene mutation or chromosomal aberration His arguments have not been generally accepted. Usually, the grounds for rejecting them is stated to be the unreality of the 'unbridgeable gaps' (e g Mayr 1942) which it is claimed, are no different in kind to the distinctions between species belonging to the same group of closely related forms It is however, not easy to deny that some of the discontinuities between natural groups seem, at first sight, to be more profound than others It is difficult to attach much meaning to arguments as to whether or not they differ in kind, unless, indeed one is really basing the discussion on the question of whether there is, or is not some novel type of 'systemic mutation' which brings them into being (cf p 62)

Goldschmidt has not been able to make any concrete suggestion as to the nature of the systemic mutation which he postulates, and [illegible]

dual genotype, but of the structure of the gene pool [illegible] represented, in fact, as the movement of the population from one general elevated region to another This will, of course, involve considerable changes in gene frequencies and perhaps in their

arrangement in the chromosomes But in so far as the essence of the process is to shift the population over a valley of unfitness into a new region of Wright's model, it is to be explained by some special type of selection, rather than by a peculiar manner of gene or chromosome mutation The basis of such a radical alteration of the organisation of the gene pool may well be, as Goldschmidt suggests, the appearance of a 'hopeful monster'—a completely abnormal phenotype which yet has selective value in some environment open to it But there is no absolute necessity to suppose that the hopeful monster is brought into being by any-

'tuning' which is discussed on p 183

Within a population which is undergoing only gradual evolutionary change, and is not in process of making a leap from one major adaptive peak to another, we can discuss the variations of fitness in somewhat simpler terms We are often interested not only in alterations in the individual as a whole, but in the evolutionary fate of particular characters of it If any character served only a single purpose in the life of the organism—or perhaps it would be better to say, fulfilled only one function—it would be possible that there would be no optimum level in its development, and that fitness would continue to increase however far the character progressed beyond the norm, provided it changed in the right direction But in fact such situations probably never occur Every feature of an animal is involved in several different functions, or at least is inter-related with several different aspects of the organism's metabolism Bones are not only structural supports, which should be strong, but must also be moved, which can be more easily accomplished if they are light, an enzyme which produces some important substance can do so only by depleting supplies of substrates which could be used in other ways Thus all characters must be subject to a balance of selective forces Too great an alteration in one direction, although in itself favourable with respect to one function, will be disadvantageous

with respect to another. It is for this reason that we may expect there to be, for any particular population over an evolutionarily short period of time, an optimum degree of expression of each and every character. Such an optimum can only be transcended if there is a qualitative, rather than a merely quantitative, alteration in the character Thus if the bones of a vertebrate limb become thicker, they certainly become stronger but after a certain limit is passed they also become inconveniently heavy, but this dilemma could be avoided if their structure were altered in some way which gave them a greater strength for the same weight

It might at first sight seem possible to classify the circumstances which lead to the existence of optima into two categories On the one hand, there would be those situations in which an increase in some aspect of the character inevitably leads to disadvantageous changes in some other aspect, while in the other class we would put situations in which the association with harmful effects seemed to be fortuitous Thus an increase in body-size, while advantageous from the point of view of strength, might seem necessarily to entail the disadvantages of lesser mobility and greater demands on foodstuffs, *while on the other hand, it is not easy to see that* any inescapable harmful effects arise from, say, an increase in the number of abdominal bristles in *Drosophila* Such a distinction cannot, however, be maintained in any rigorous manner The example of the bones, mentioned above, shows that *it is unjustifiable* to suppose that there are any cases in which the selective forces whose balance produces the optimum are so *indissolubly* ... ble There ... in the ... gative selection pressures Increased body size *leads to lower mobility* only if the body structure remains unaltered *and to an increased* demand for food only if metabolic efficiency is not improved Thus, in the last analysis, the association of disadvantages with the continuance of a type of alteration which in its less extreme manifestation is useful to the animal, is always a contingent one, and never inevitable

Characters may differ in the kind of optimum which they

exhibit If one plots fitness against some measure of the character, a curve will be obtained which has a peak at the optimum This may be called the fitness cross-section of the character (cf Fig 8) It may show a rapid loss of fitness with quite small variations from the optimum or the character may be much less crucial for survival The difficulty of deciding on what scale to measure the character arises here as it does in connection with assessing variability and canalisation (see p 138)

Before proceeding further with the argument, it is necessary to distinguish between two types of 'fitness cross-section' which may be associated with a character In the first place, one may consider variations in fitness which arise from a direct causal relationship between the character and the environment and mode of life of the organism For instance, in terms of one of the previous examples, we would expect that fitness would be lessened if the bones of an animal were either too heavy or too light The curve expressing this relation might be called a 'direct' or 'true' fitness cross-section But genes, or environmental stimuli, which tended, say, to lighten the bones, would probably have side-effects on other aspects of the organisms, these being known as pleiotropic effects when it is a gene which is in question Within any given population, or range of environmental conditions, there will only be restricted numbers of genetic or other stimuli which are available to produce lightening of bones, and it may be that these will have the property that their side effects on something other than bones always play an important part in determining fitness In that case, if we were able to measure the relation between bone-thickness and fitness, the curve we would obtain would not express the direct phenotypic effect of the skeletal character, but would be a spurious fitness cross-section, arising largely from pleiotropic effects (Fig 15)

There is no doubt that in many situations the fitness cross-section which is most easily noticeable in experiment is actually spurious For instance, Clayton, Morris and Robertson (1957) selected *Drosophila* for the number of bristles on certain abdominal segments The selection was repeated, as identically as possible, in a number of lines originated from the same base population There

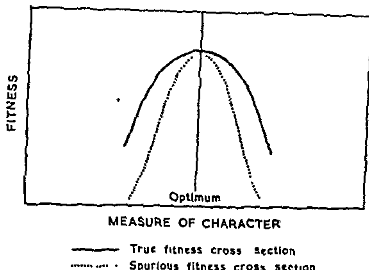


FIGURE 15
True and spurious fitness cross-sections of a character.

was considerable, though perhaps not extravagant, variation in the degree of response of the various lines (see Fig. 16). From the present point of view, the important thing is what happened to the lines when selection ceased and they were left to the mercy of the natural selective forces which must operate in the comparatively crowded conditions of the cultures. It will be seen from Fig. 16 that all the lines selected for high numbers of bristles showed some decline in bristle number when selection was relaxed, which must indicate that the individuals with high numbers were less fit than those with lower numbers. However, it is apparent that their fitness, or lack of it, is not a direct consequence of the *number* of bristles they bear, since the highest lines (Nos H1, H5) do not fall back much faster than the lower ones; and indeed after 19 generations, when natural selection must have almost achieved its full effect, these two lines have only sunk back as far as a point which, for line *H4*, was one of instability.

number to fall further towards that of the base population, it

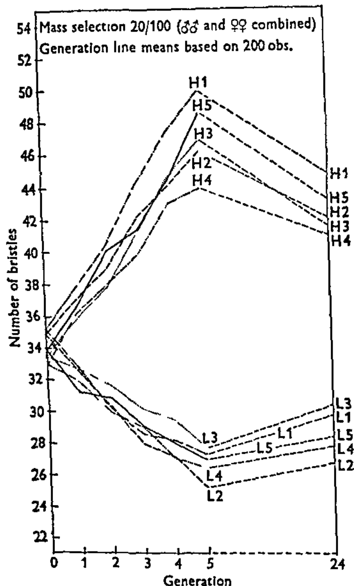


FIGURE 16

Selection for number of abdominal bristles in Drosophila Five upward and five downward selection lines were carried to the fifth generation, selecting the extreme 20 out of a sample of 100 Artificial selection then ceased, and the lines were left to natural selection for the next nineteen generations (From Clayton, Robertson and others 1957)

natural selection had tried to push them there One must conclude that the degree of unfitness of the selected lines, which led to their fall when selection was relaxed, was not due to bristle number as such, but to *pleiotropic effects* of genes which had become concentrated during the selection, and which were got rid of by natural selection at the cost of only a moderate fall in bristle number

The existence of such spurious 'pleiotropic' fitness cross-sections makes it difficult to find any precise method of arriving at the true form of this curve Nevertheless, there can be no doubt that some phenotypic characters of an animal may have a direct causal effect on fitness, and, even if difficult to measure, the true fitness cross-section remains a valid theoretical notion So far as the characters of an organism are moulded by natural selection, they will be controlled by the forms of their true fitness cross-sections In any particular population, some characters (such as for example, abdominal bristle number) may have a fairly definite value which is dependent on a spurious fitness cross-section, but if this is so, the character cannot be said to be moulded by natural selection, which controls it only at second hand If the value of the character could be altered leaving the rest of the organism unchanged, the alteration would be indifferent as far as natural [illegible] concerned Moreover the spurious cross-section could

[illegible]

it is always the true, not the spurious curve which is meant

2 *Canalisation of the phenotypic optimum*

[illegible]

lar character to exactly the optimum degree Firstly the population might contain no variation in genes affecting the character, or secondly, the epigenetic processes occurring during the development of the organism might be so buffered or canalised that the optimum end-result *is* produced irrespective of the genes which the individual contains

In practice neither of these alternatives can be completely

realised The complete absence of genic variation is impossible, firstly, because mutation cannot be wholly suppressed, secondly, because no environment is ever wholly uniform and a population containing no genic variation has no reserve to cope with environmental changes and does not contain any potentiality for further evolution, and thirdly, because homozygotes seem usually to be more phenotypically variable in an inconstant environment than the heterozygotes are, and therefore provide an unreliable mechanism for attaining an optimum condition Similarly, complete canalisation of development is impossible in practice, firstly, because it provides no mechanism by which the phenotype can be altered so as to adapt it to a changing environment, and secondly, because, by eliminating phenotypic variation, it leaves nothing on which natural selection can operate, and therefore brings evolution to a full stop

In any real population, therefore, the attainment of, or approximation to, the optimum value for a character will depend on a combination of these two methods, neither being carried to completion We shall have in the first place some limitation on the amount of genic variability contained in the population This can be achieved by the elimination, or reduction in frequency, of certain genes by natural selection against them In a relatively constant environment, which varies only over a certain limited range, natural selection of this kind will tend to maintain an equilibrium set of gene frequencies This will amount to a 'genetic homeostasis' The type of selection by which this is maintained is that most usually referred to when the term 'natural selection' is used And it has been suggested (p 72) that as a more precise name for it one might use the expression 'normalising selection'

At the same time the epigenetic system would be rendered more or less unresponsive to environmental or genetic variations in such a way that it tends to produce the optimum, or something close to it, even when the environment or the genotype of the particular individual deviates fairly widely from that normal for the population. The nature of the processes concerned in this canalisation are not known in detail, but there is nothing very

natural selection had tried to push them there One must conclude that the degree of unfitness of the selected lines, which led to their fall when selection was relaxed, was not due to bristle number as such, but to pleiotropic effects of genes which had become concentrated during the selection, and which were got rid of by natural selection at the cost of only a moderate fall in bristle number

The existence of such spurious 'pleiotropic' fitness cross-sections makes it difficult to find any precise method of arriving at the true form of this curve Nevertheless, there can be no doubt that some phenotypic characters of an animal may have a direct causal effect on fitness, and, even if difficult to measure, the true fitness cross-section remains a valid theoretical notion. So far as the characters of an organism are moulded by natural selection, they will be controlled by the forms of their true fitness cross-sections In any particular population, some characters (such as for example, abdominal bristle number) may have a fairly definite value which is dependent on a spurious fitness cross-section, but if this is so, the character cannot be said to be moulded by natural selection, which controls it only at second hand If the value of the character could be altered leaving the rest of the organism unchanged, the alteration would be indifferent as far as natural selection is concerned Moreover the spurious cross-section could easily be altered by the selection of the general genetic background In the future discussion, when fitness cross-sections are referred to, it is always the true, not the spurious curve which is meant

2 *Canalisation of the phenotypic optimum*

In theory, there are two alternative ways in which it could be arranged that the individuals in the population develop a particular character to exactly the optimum degree Firstly the population might contain no variation in genes affecting the character, or secondly, the epigenetic processes occurring during the development of the organism might be so buffered or canalised that the optimum end-result is produced irrespective of the genes which the individual contains

In practice neither of these alternatives can be completely

development against environmental variations It seems probable that this has often been the situation in wild populations, in which it is found that many characters have a very well defined wild-type which shows remarkably little phenotypic variation under a wide range of environmental conditions If, on the other hand, the epigenetic system is such that an environmental change tends to modify the end-state of development in the same direction as the optimum shifts, then it will clearly be advantageous not to buffer development so strongly that this direct adaptive change no longer occurs Canalisation will only be encouraged so far as is necessary to adjust the magnitude of the effect of the environment on the phenotype to the change produced in the optimum. The intensity with which natural selection will operate in either of these two cases is, of course, dependent on the third factor mentioned above, namely the degree of disadvantage associated with a certain phenolypic deviation from the optimum

Selection for epigenetic canalisation has been referred to as 'stabilising selection', but this term should not be taken to imply that the maximum selective advantage would ever lie with complete stabilisation A better name would be 'canalising selection' Within the general category of canalising selection one can, as we have seen, distinguish between selection for canalisation against genetic variations, and selection for canalisation against environmental variations

The discussion above has implied the existence of another type of selection, namely selection in favour of epigenetic systems which respond to environmental changes by producing phenotypes modified in the same direction as the optimum has been altered This may be called 'selection of adaptability'.

As an alternative to the evolution of epigenetic canalisation it might be suggested that a population can carry a reserve of genetic variability within itself without harm, provided that some genetic mechanism can be found for keeping together groups of complementary factors which are so balanced as to produce the optimum phenotype Mather (cf 1943) has suggested that linkage might provide such a mechanism, the factors which would tend to cause the phenotype to deviate from the optimum in one

direction normally occurring in the population linked to factors of the opposite tendency. However, crossing-over would continually *tend to break up such linked complexes* *Properly balanced* groups can only be the prevalent type in the population if selection eliminates the ill-balanced complexes as fast as crossing-over makes them Some species have evolved a genetic system which contains many chromosome inversions, which act as crossing-over suppressors and thus preserve well balanced complexes of complementary factors from disruption Many well studied species of *Drosophila* fall into this category, a circumstance which often tends to make the suggestion of linked complexes seem rather *plausible as a general mechanism for stabilising the phenotype at* an optimum level But as Sewall Wright (1952) has shown, in normal diploid organisms without inversions, it is only extremely short linked groups which would keep together, unless indeed the selective values of the balanced and unbalanced *combinations* of genes differed by an improbably large amount The postulated linked complexes would, in fact, have to be little larger than groups of pseudo-alleles, and the breakdown of a balanced complex by crossing-over becomes almost indistinguishable from *the origin of a new genetic variant by mutation* *It seems unlikely* therefore that, except in the special case where inversions are involved, linkage plays a major part in the organisation of the gene pool. Moreover, it obviously cannot be invoked to account for the stability of a character when this is known to be affected by genes on different chromosomes, as most characters in fact are

The major feature of a canalised system is, of course, the fact that there are alternative developmental pathways, *separated by* 'thresholds', and leading to qualitatively different end-results *This consideration sets the general* framework within which we can discuss the nature of the 'canalisation cross-section' (p 34) of a particular character In general terms, we wish to consider the relation between the degree of phenotypic expression and the measure of the stress applied to the epigenetic system by abnormality either in environmental or genetic conditions *In theory* one could plot the value of the phenotypic character against the dosage of some gene-produced *active agent* (which may be the

gene itself) If the wild type is highly canalised then in the neighbourhood of the wild type gene dosage we should find that comparatively large variation in dosage produces little change in phenotype (Fig 17) Similarly, if the phenotypic measure is

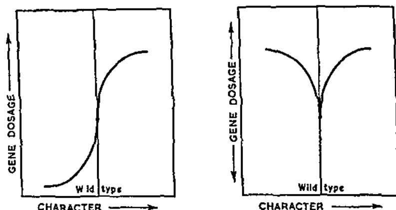


FIGURE 17

ordinate corresponds to the cross-section of an epigenetic pathway

plotted against some environmental variable, we should find that in the environmental range against which the epigenetic system is buffered, large changes in the environment produce little change in the phenotype Another way of representing the same facts is reached if we turn one half of such a curve upside-down For instance, suppose that the phenotypic measure is plotted horizontally A certain point along this axis will represent the wild type phenotype and thus will correspond to a particular gene dosage In the region in which the values of the phenotypic character are greater than in the wild type, the vertical scale will represent increasing dosages of genes tending to increase the character In the region in which the phenotypic values are less, we can use the same vertical scale to represent the *decreasing*

quantities of gene dosage of the same kind We shall thus convert the continuously rising (probably sigmoid) curve which would appear on a normal plot of gene dosage against effect into some form of U-shaped curve which at first falls and then rises again (Fig 17)

Such a curve can be regarded as an intercept between a time horizon (the adult state) and a probability surface which is a function of the epigenetic system One can take it as indicating the state to which the epigenetic system tends to move (the lowest point in the curve), and the degree of constraint which has to be applied to the system to force it to achieve some other end will be shown by the slope of the curve on each side of the lowest point This constraint can be applied either by genetic variations (i e differences from the average genotype of the population) or by environmental variations (differences from the average environment of the population) Such a diagrammatic way of symbolising the situation cannot, of course, be pressed too far Even if we are considering one and the same phenotypic character, all the genetic variations affecting it cannot be truly represented on a single axis as though they were all reducible to dosages of a single variable The same is true of the various types of environmental variation which may occur (p 38) Moreover the epigenetic canalisation against genetic variation may not be exactly the same as that against environmental stimuli, although the two are probably always fairly closely connected Nevertheless such a semi-diagrammatic symbolisation does enable one to form a picture of some of the more important qualitatively different types of canalisation which may be found in different circumstances

value, at which it comes to a sharp point This has been referred to (*Waddington 1952a*) as 'cusped' canalisation (Fig 18) We should find in such cases that a considerable change in gene dosage or in environmental conditions is needed to produce any phenotypic divergence away from the wild type In an animal such as *Drosophila* there are obviously very many characters for

which this sort of situation occurs, for instance, the wing venation, the position and number of the major bristles, etc The wild type may, in fact be protected by quite strong thresholds, so that considerable genetic or environmental changes are necessary before any phenotypic alteration is produced at all In all such cases of cusped canalisation the slope is bound to decrease for some

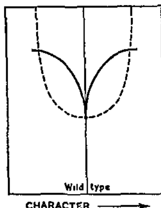


FIGURE 18
Cusped canalisation (solid lines) *and 'smooth canalisation* (dashed lines)

time as we move away from the wild type value In the regions of lower slope a given change in dosage of gene or in environmental conditions will produce a larger phenotypic alteration The phenotypic variance may then be expected to be larger in a population with this average gene dosage, or living under this average environmental condition, than it is at the wild type position

In contrast to such cusped canalisations we may have examples of 'smooth' canalisations, in which the curve is U-shaped, with the more or less horizontal portion in the neighbourhood of the wild type value (Fig 18) In such cases small genetic or environmental *variations around the mean produce quite considerable* phenotypic effects, but it becomes progressively more and more difficult to obtain extreme phenotypes This must be the situation

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fattening or stunting occur For some other characters, such as the rate of attaining sexual maturity, the thresholds protecting the 'normal' may be even more strongly marked Again, in poikilothermic animals there may be a wide range of temperatures in which development proceeds along its normal course, from which it can only be deflected by rather intense stresses, although in the same organism the rate of development may show only a smooth and much less strong canalisation, if indeed it exhibits any at all

It is not easy to quote diagrammatically clear examples of canalisation against variations in gene dosage Ideally, one should have e idence of the phenot [illegible]

c

b

that kind is that of 'bobbed' in *Drosophila melanogaster*, studied by Stern (1929) The curve relating gene dosage to effect was of [illegible]

there was no upper range of doses within which the epigenetic system again became sensitive to additions, or at least if there was Stern did not reach it We can express the situation by saying that the length of the bristles has a very asymmetrical canalisation against the dosage of bobbed genes, there is a steep threshold preventing them getting longer than normal, a less marked one inhibiting them from becoming shorter

There is no reason to suppose that the bobbed situation is anything other than the normal one The difficulty in providing similar evidence in other cases is simply the technical one that it is only in special situations that one can add or subtract single genes to an otherwise unaltered genotype In case of bobbed this can be done because the gene lies in the Y chromosome, which is relatively inert For genes in other chromosomes, we have indicative evidence for the existence of similar situations For instance, the phenotypic effects of compounds of the white series

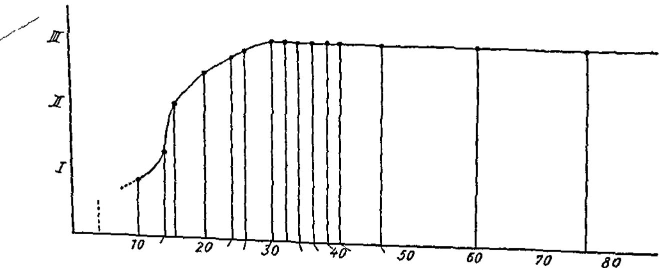


FIGURE 20

Different doses of 'bobbed' alleles The length of the bristles is shown vertically and the dose of gene-activity horizontally (After Stern 1929)

in *Drosophila* would lead one to suppose that there also the wild-type has a strong threshold preventing the eye becoming darker than normal, while the existence of two or more 'wild-type' iso-alleles with different degrees of dominance shows that phenotypic normality can be produced by a range of effective gene dosages

The epigenetic feed-back mechanisms on which canalisation depends can, of course, be regarded as examples of gene interaction. Interaction between two allelomorphs is referred to by such terms as dominance, recessiveness, over-dominance, etc Interactions between different loci come under the heading of epistasis This is perhaps most usually thought of in terms of interactions between only two or three loci We know, however, that in the development of any one organ very many genes may be involved, and in canalised epigenetic systems we are probably confronted with interactions between comparatively large numbers of genes Extremely aberrant phenotypes are then likely to be produced by rather special combinations of allelomorphs of different loci The genotypes producing them can then be compared to good hands of cards at a game such as Bridge or Poker, rather than to large totals on some single scale of dosage In so far as this analogy is valid, we may then expect that when two extremely aberrant phenotypes are crossed the progeny will tend to revert to the mean of the population, just as two good Bridge hands shuffled and redealt are rather unlikely to give rise to equally good hands Again this process of reversion may be asymmetrical In Bridge a mixture of a number of good hands can revert towards the average more easily than a mixture of a number of bad hands, since all the bad hands will contain few picture cards and a mixture of them is likely therefore to remain rather bad. Only empirical investigation of each particular case can show what its genetic situation is in this respect

3 *Inter-relations of the factors*

In a population in the wild, natural selection will be operating, and will impinge in the first place on the developing phenotypes, both juvenile and adult, which are offered to it. A full understanding of the situation therefore requires a consideration of how

these phenotypes are produced, and thus of canalisation, as well as of the gene-frequencies The orthodox population-genetics of existing mathematical theory often seems to confine its attention to gene-frequencies, but hidden within all such theories are certain assumptions about dominance-recessiveness or additiveness of gene-action, it is these assumptions which require fuller discussion in terms of canalisation And it will also be necessary to develop the concept of the environment somewhat further than is usual

The structure of a population is affected by a number of factors, of which the most important are the following

(1) The environment This may be defined, in the context of evolution, as the totality of circumstances physically exterior to the organism which affect its reproductive contribution to the next generation For the purposes of mathematical treatment, it is often taken as being the same for all individuals in the particular population under consideration This is probably always an over-simplification, and may often be an unwarranted one It is clear that the environment does not normally remain the same from moment to moment throughout the lifetime of any one individual, varying for example in such factors as temperature, humidity, the attack of predators, etc Unless all individuals of a generation are strict contemporaries, these temporal fluctuations in the environment must affect different ontogenetic stages and thus will have differential effects on the individuals exposed to them Again, the environment is rarely completely uniform in space, except perhaps in the sea, and this inhomogeneity also will not fall with the same incidence on all individuals

In the consideration of many types of population change, particularly those which are of essential importance in the evolutionary divergence of species, we need to take account not only

tions it actually utilises, but by what might be called an [illegible] ence' This might be defined as the environment which the population could utilise, given an evolutionary adaptive change of a magnitude which could occur in a number of generations small in comparison with the number necessary to convert the population

into a new species The ambience of many species is much wider than their actual environment That of a hedge-dwelling bird, for instance, includes the open fields and the tree-tops, and any naturalist would be able to quote innumerable detailed examples In spite of the hypothetical element which enters into the definition given above, the ambience is a real factor in the evolution of a population—one which is taken advantage of whenever a group migrates into new territory or begins to occupy a new ecological niche One could, in fact, probably define the concept equally appropriately in a less hypothetical manner, as the totality of all environments which aberrant individuals of the population attempt to utilise

(ii) A second factor affecting the population structure is the direct effect of the environment on the phenotypes This may be deleterious, in the sense of lowering reproductive efficiency, as it is for example when cattle of the temperate zone are taken to the tropics In many cases, however, the effect is advantageous, development being modified so as to result in an adult phenotype which is particularly adapted to the environment which has been acting It is clear that the genotypes which natural selection will favour are those which carry with them a capacity for such adaptations

(iii) The array of gene frequencies If the environment were uniform, and the same for all individuals, one might, naively, expect that the whole population would come to have the same, optimum, genotype except in so far as recurrent mutation maintained a pool of rare mutant alleles This is, indeed, the picture which most geneticists held a quarter of a century ago We know now that it is very far from representing the actual state of

[illegible]

heterozygous and that the gene pool of a population contains different alleles at very many loci This is so even in populations which are in some degree of equilibrium, and are not actively changing their habitat or manner of life

One of the main principles which is invoked by modern

population genetics to explain the persistence of several alleles is the theorem that more than one allele will be preserved only if there is selection in favour of the heterozygote (Haldane 1927 Fisher 1930) Some authors (e g Ford 1953) appear to suggest that it is the only mechanism by which genetic diversity can be permanently maintained (except for the special case of the genetic determinants of outbreeding systems such as sex) However, this is true only if the environment is supposed to be the same for all members of the population If the environment fluctuates in time, or varies in space, so that the selective value of one and the same genotype is not the same for all individuals in which it occurs, genetic diversity may be preserved even if the heterozygotes are not superior to the homozygotes in any of the niches (Levene 1953, Li 1955) Such an environment may be referred to as '*partitioned*' *The chance of a stable equilibrium between* different genotypes is still greater if the animals show habitat-preferences or non-random mating (cf Waddington, Woolf and Perry 1954 and p 108)

From the point of view of population structure, the most immediately important aspect of the diversity of genotypes is *not* the actual number of different alleles present, but rather the variation in the total 'dosage' of gene-activity affecting the character under consideration Suppose there are a number of loci contributing to a certain gene-activity Q, and that the phenotypic optimum is produced by a dosage of activity q, then two populations each with a variance of gene dosage of $\frac{1}{2}q$ will appear much *the same* whether this variation is dependent on numerous alleles at many loci, each having small effects, or on a smaller number of alleles of more marked effect The two populations will, however, respond very differently to selection the former *moving more* readily to a position far from the original situation

(iv) The degree and type of *canalisation* of the various charac- [illegible] members will

amount of genetic variation which can remain [illegible]
partially 'hidden'

(v) The *final factor which we need to consider* is the degree of

selective disadvantage associated with any given divergence from the optimum phenotype For some populations, the environmental circumstances may be 'permissive', that is to say, they may exact only a slight penalty for even quite considerable abnormality, for others, they may be much stricter According as the fitness cross-section of the character falls steeply or slowly on either side, one could speak of the optimum being 'narrow' or 'broad', or the environment as being 'restrictive' or 'permissive' It should also be remembered that there is no reason to suppose that the curve relating selective value to phenotype need be symmetrical, divergences on one side of the optimum may have much less deleterious effects than on the other

In any population, these five variables will react upon one another in a rather complex manner The general framework of these interactions is suggested in Fig 21 The arrows indicate

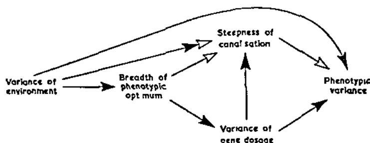


FIGURE 21

The inter-relations of the five main factors which determine the organisation of the gene pool A solid arrow leading from one variable to another indicates that when the first increases, this tends to cause an increase in the second an open arrow indicates the opposite relation

causal relations Those with a solid arrow are direct relations, those with an open one inverse Thus, in general, the greater the diversity of the environment, the broader the phenotypic optimum and hence the greater the variance in gene dosage, and finally the phenotypic variance At the same time, if there is a great variability in genotypes there will be a tendency for the

evolution of a steeper canalisation which will conceal its phenotypic effects On the other hand, the broader the phenotypic optimum, the less steep the canalisation Variation of the environment will act directly to increase the diversity of the phenotypes On the canalisation, it may operate in either of two opposite ways In so far as the direct effect of environment on phenotype is deleterious, an increase in the variability of the environment will tend to be followed by an increase in the steepness of canalisation, but if alterations in the environment produce adaptive changes in phenotype, then the canalisation will not be so far steepened as to render such changes impossible—in fact, as we shall see, the canalisation will be adjusted so as to further them.

It is clear that, on this basis, a population has too complex a structure to be amenable to *a priori* argument. In particular, it is difficult to decide, when considering two populations, whether a given character may be expected to be more steeply canalised in one or the other The only sure guide is the empirical test of experiment. The buffering against environmental influences can be fairly easily studied by an investigation of the phenotypic responses of the two populations to changes in environmental conditions

[illegible] might

canalisation The canalisation cross-section of a character would then directly represent the phenotypic effects of variations in the effective dosage of the genes acting on it. One would expect it to be rather closely related to the fitness cross-section and in fact to be, on the appropriate scale, the inverse of it. For consider a given phenotypic deviation from the optimum, the greater the deficiency of fitness associated with this, the stronger will be the selective pressure to prevent its occurrence, that is to say, the tighter will be the canalisation so that a larger change in gene dosage will be required to bring about so great a deviation in development. Thus for characters for which there is a well marked wild-type, deviations from which are strongly deleterious, we should expect that the normal course of development would be

protected by appreciable thresholds Gene action in the neighbourhood of such a wild type will be far from additive; in fact at most loci dominance will be important, and there may be strong epistatic interactions operating to suppress aberrations from normality of phenotype In systems of this kind, gene action may tend to be more nearly additive when we are dealing with a phenotypic range some distance away from the normal optimum, this seems to be the case with the abnormalities of the crossveins in *Drosophila* mentioned on p 139 On the other hand, if the fitness cross-section in the neighbourhood of the optimum is such that the commonly occurring aberrations are unimportant there will be little selective advantage in buffering development in this range against minor genetic variations, and we might well expect to find that gene action was more or less additive There is clearly a problem here of deciding on the scale by which gene dosage should most appropriately be measured The selective pressure towards canalisation will only be effective if the genetic *variance present in the population tends to throw up phenotypes* sufficiently far removed from the optimum to suffer appreciable disadvantages We need, therefore, to consider the fitness cross-section of the character plotted against units of gene dosage defined in terms of the available gene variation Further gene action can only be expected to be additive when the slope of the fitness profile remains constant as one goes away from the optimum, if the profile has an appreciable second differential coefficient, the curve relating gene dose to phenotypic effect will not remain linear

It is difficult to be sure, at this stage, how far these predictions are fulfilled, since it is only rarely that we have any independent evidence as to the selective importance of the various characters whose population genetics has been studied Robertson (1956) has suggested that additiveness, in the neighbourhood of the optimum, should be found for characters which play little part in determining fitness He has mentioned the number of abdominal bristles in *Drosophila* as a possible example, contrasting it with such characters as numbers of eggs laid and percentage larval survival, which are of obvious selective importance, and

for which gene action seems to be much less nearly additive We have, however, not even an approximate understanding of the fitness cross-sections of these characters, and these suggestions remain only the first tentative gropings into this highly complex field It must be remembered, also, that the arguments we have been considering have left out of account the influence of the environment on the phenotype, by way either of deleterious aberrations from the optimum or of advantageous exogenous adaptations, both of which must affect the canalisation, that is to say the additiveness or otherwise of gene action

One is often tempted to try to compare the buffering of one character with that of another One tends to think of a character which shows very little variation as more highly canalised than one which varies to an obvious extent It is, however, as difficult to attach a precise meaning to a comparison of the canalisations of two characters as it is to that of their variances, since there is no obvious common scale to use in assessing them One can at best choose some appropriate relative method of expressing the magnitude of the variation. For instance, one might measure the range of variation of a character in a given population in terms of the total range which it exhibits in all populations of the species or one might compare it with the range of variations found when nearly related species are also brought into the picture, or one might take the physically possible range as the standard of comparison

In some such terms, it may be possible to make comparisons between the variances of different characters, and thus of their degrees of canalisation, in terms which are meaningful in some particular context. To take a fairly clear example, it might be found that the variation between the numbers of sternopleural hairs on the two sides of a series of single individuals of *Drosophila* was a large fraction of the variation between individuals while the intra-individual variation in wing length was only a small fraction of the inter-individual Then one would have little hesitation in saying that the development of wing-length is in a meaningful way, less variable than that of the sternopleurals, and one might expect that it would prove to be better buffered

Again, in a population living in a non-uniform environment, one character (for instance, body size) may exhibit a range of variation which is a large fraction of the total range which can be elicited by selection, while another (e g a number of scutellar bristles) may show little or no variation Again, one tends to speak, loosely but not without all meaning, of the former as a more variable character than the latter Perhaps the only characteristic of a canalisation which can, strictly speaking, be used when one wishes to compare one character with another is whether it is cusped or smooth No change in the scale used for measuring the variation, applied uniformly over the whole range, will change one of these types into the other

Few attempts have been made to study the genetic control of characters which normally exhibit little variation, and for which the existence of a cusped canalisation might be suspected One character which has been investigated is the posterior cross-vein in the wings of *Drosophila melanogaster* This runs between the fourth and fifth longitudinal veins The physically possible range of variation extends from a complete absence of the vein to a supplementation of it by more or less extensive extra veins In most normal populations, living under heterogeneous environmental conditions, only a minute fraction of this possible variation actually occurs, nearly all flies have complete cross-veins, while a fraction of a per cent may exhibit a slight gap in the vein, or a small piece of extra vein However, special stocks can be built up by selection in which the vein is partially or completely missing, or in which there are larger or smaller supplementary extra veins The genetic analysis (Waddington 1955) employed four such stocks, one characterised by large gaps, one by small gaps, one by small additional veins and one by larger additional veins It revealed a rather complex situation, which it is unnecessary here to describe in detail One important point was that it became clear that a number of flies, all of wild-type phenotype but bred from different parentage, may possess genotypes which differ considerably, *and further that this cannot be explained* simply by the supposition that some of the genes concerned are fully dominant We have to conclude that the flies differ in the intensity

of the relevant primary gene-activity, but that the buffering of the epigenetic system is such that a considerable range of such activities still leads to the production of the wild phenotype The cross-vein therefore exhibits a cusped canalisation (As a matter of fact, a more detailed consideration of the situation shows that the development of the cross-vein depends not on a single process which is canalised in a cusped manner, but on the combination of a number of processes, each with this type of buffering) On the *other hand, when, in a given series of matings, genotypes are* produced in which the gene dosage is beyond the capacities of the canalisation to conceal and which therefore give rise to abnormal phenotypes, it was found that these phenotypes reflect the dosage of gene activity not too inadequately, the gene action within this phenotypic range was more nearly additive

CHAPTER 5

THE SURVIVAL OF THE ADAPTABLE

DURING THE recent war, engineers attained some facility in designing machines to carry out tasks which earlier generations would have considered beyond the capacities of anything but an intelligent being One example of such a mechanism was the automatic predictor gun-sight In this, radar signals were emitted, the reflections of these from any aircraft in the neighbourhood received, and the information thus provided as to the aircraft's position, direction of motion, speed, etc , fed into an electronic apparatus which calculated the position in which the aircraft might be *predicted to be at a given time later, and at the same* time controlled the position in which a gun was pointing in such a way that when it was fired the shell would exactly reach this position Those who had much to do with these machines, or who watched the results of their operations, learned that the claims of engineers, like those of others, go down more easily if taken with a pinch of salt But whether such guns hit their targets or not, they certainly seem to have struck, forcibly and frequently, the imagination of theoretical biologists. In fact the irreverent may be tempted to feel some amusement that mechanisms of such widely advertised infallibility, set to the task of shooting down German bombers, should at the end of the war exhibit a bag containing so many neurologists, sociologists, psychologists

One of the authors who has used such mechanisms to illustrate his thought is Dr G Sommerhoff, whose book *Analytical Biology* is largely concerned with the concept of adaptation This is widely recognised as one of the focal ideas of biology. It indicates one of the major problems that has to be dealt with by

the theory of evolution, it is a central feature of physiological functioning, and, in the wide sense given it by Sommerhoff it embraces the whole of the regulatory aspects of development. Much of Sommerhoff's work is devoted to searching for an adequate definition of the concept, which will give it a precise meaning in terms which are unexceptionable even to the severest non-biological critic. It is as a paradigm for the essential features of adaptation that he takes the example of the automatic predictor gun-sight. He analyses this situation into four elements. One is the responder, the thing adapted, the gun. This adapts to the second factor, namely, the relevant part of the environment, that is the present position of the aeroplane. This adaptation is with respect to a third element in the situation, namely the goal—in this case the shell hitting the target. The fourth component in the situation is what Sommerhoff calls the coenetic variable. This is a prior condition which has a causal connection with both the environmental situation, which is adapted to, and the responder, which adapts, these two being jointly affected in such a way that they issue in the goal (or, as he also calls it, the focal condition) of the adaptation. For the gun-sight, the coenetic variable is the earlier movement of the aircraft, which not only determines its position at the later time when it acts as the environmental situation but also, through the reflected radar waves, determines the position of the gun.

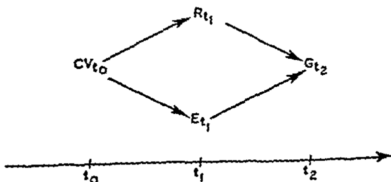


FIGURE 22
Sommerhoff's definition of adaptation (From Sommerhoff, 1950)

With this as an analogical basis he proceeds to define a new concept, that of directive correlation, which, he says, 'will become our main instrument for expressing in precise terms the various forms of purposiveness found in nature'. The definition (see Fig 22) runs as follows

Definition Any event or state of affairs R_{t_1} occurring at a time t_1 is directively correlated to a given simultaneous event or state of affairs E_{t_1} in respect of the subsequent occurrence of an event or state of affairs G_{t_2} if the physical system of which these are part is objectively so conditioned that there exists an event or state of affairs CV_{t_0} prior to t_1, and a set of possible alternative values of CV_{t_0}, such that

(*a*) under the given circumstances any variation of CV_{t_0} within this set implies variations of both R_{t_1} and E_{t_1},

(*b*) any such pair of varied values of R_{t_1}, E_{t_1} (as well as the pair of their actual values) is a pair of corresponding members of two correlated sets of possible values R'_{t_1}, R''_{t_1}, R'''_{t_1} and E'_{t_1}, E''_{t_1}, E'''_{t_1}, which are such that under the circumstances all pairs of corresponding members, but no other pairs, cause the subsequent occurrence of G_{t_2}

Our previous analysis of adaptation shows that every adaptation is an instance of directive correlation, but not vice versa Directive correlation is a slightly wider concept. For inspection of the usual meaning of 'adaptation' shows that in the case of an organism-plus-environment system the coenetic variable consists always of an environmental variable and, in fact, usually of an antecedent value of the environmental variable to which a given response is regarded as adapted (cf §13) On the other hand, directive correlation, as defined above, contains none of these restrictions concerning the coenetic variable

He therefore defines adaptation as a slightly more limited concept than directive correlation The statement that an event or state of affairs X_{t_1} is adapted to another event or state of affairs Y_{t_1} from the point of view of some future event or condition FC_{t_2}, means that X_{t_1} and Y_{t_1} are connected by a directive

correlation which has FC_{t_2} as focal condition and in which a prior value of Y, say Y_{t_0}, acts as coenetic variable

There are several points to be made about these definitions In the first place it may be pointed out that the inclusion of *coenetic* variable in the definition in effect begs the question as to the nature of adaptation It insists that adaptation arises as a consequence of some factor prior to the situation under observation, which causally *influences both the thing which adapts and that* which it adapts to, in such a way that the goal of the adaptation is attained This excludes by definition the possibility of any vitalistic explanation of adaptation Even though one wishes to reject such explanations, it is well to remember that the coenetic variable is not in general a fact of observation, it is something for which we have to seek, and have, indeed, in most cases not yet discovered The definition is not a straightforward ostensive one, which indicates a phenomenon, but involves a theory as to the way *in which the facts are to be explained*

It is perhaps more important from my present point of view to ask whether the concept which Sommerhoff has defined is that which is normally referred to when the word adaptation is used *Is he not rather speaking of adaptability, that is, the capacity* for adaptive response? In the concept as he expounds it the thing which adapts always becomes modified in a manner appropriate to the particular environmental situation, just as, each time the gun fires, it points in a direction related to the *particular movements* of the aircraft in that instance Now the normal use of the word adaptation does not necessarily involve such modifications We might say that an animal with a thick fur is adapted to a cold climate without meaning to imply that the thickness of the fur would alter *in relation* to the intensity of the frosts to which it was subjected Where such modifications in relation to the environment do occur, we would, I think, speak of a thing being 'adaptable' rather than 'adapted' For instance, we *should* say that the diameter *of the pupil of the* eye is 'adaptable' *over a* certain range of light intensity, meaning that within this range it can alter in conformity to the environment in such a way as to attain the goal of keeping the intensity of light at the *retina*

within certain limits On the other hand we use the word 'adapted' of a persistent condition which is such that within a certain range of variation of the environment the goal is attained For instance, when after a certain length of exposure to a given intensity of light the diameter of the pupil has settled down to a constant value, we might say that it is 'adapted' to that illumination We conventionally use the word 'adaptation', in fact, not precisely of the circumstances defined by Sommerhoff

The word is in fact used in two different senses, for a structure or function which is appropriate to some particular set of conditions (thick fur, or an active metabolism, is an adaptation to a cold climate), or for the process by which such a structure or function comes into existence Neither of these is exactly the concept which Sommerfeld has defined, he has described systems with the capacity for carrying out processes of the kind referred to when the word is used in the second sense (One could modify his definition of directive correlation to deal with the first sense of 'adaptation' by specifying that the values of R'_{t_1}, R''_{t_1}, etc, which occur in paragraph (*b*) are all identical)

The concept which Sommerhoff himself defined, and which I suggest should be called adaptability rather than adaptation, is probably the more fundamental idea When we say, using conventional ideas, that a thick-furred animal is adapted to a cold climate, we probably in the first place think of both the thickness of the fur and the temperature of the environment as constants On reflection it is obvious that over any sizeable section of the life history of the animal the temperature will not remain constant Although the thickness of the fur does not alter, some other aspect of the metabolism of the animal will be modified in correlation with the temperature—perhaps its rate of sweating, its heat loss through breathing, or the amount of foodstuffs consumed to produce internal warmth Thus for the animal as a whole the focal condition (i e its continued existence) is attained only because some parts of the system are adaptable over the range of *environmental variation encountered* The features of the animal which are adapted, and which do not become modified in correlation to the environment, are being carried, as it were, by

the adaptable ones If, however, the environment becomes much hotter, the temperature may pass out of the range within which the rest of the heat-regulating mechanism can cope with the thick fur, and in this case we can say that the fur is not adapted to the prevailing conditions (of which the remainder of the homoiothermic apparatus is one) Equally we could regard the heat-regulating mechanism as being ill-adapted to the situation of which the thickness of the fur is a part If the goal, the persistence of the animal, is to be achieved, one or other or both of these two elements the fur and the remainder of the heat-regulating mechanism, must be altered The alteration will consist either in a change in the adaptability of the physiological homoiothermic mechanism or a modification to the thickness of the fur If the latter occurs we should speak of it as a new adaptation Again in normally fluctuating circumstances its success would be dependent on the existence of a certain adaptability in the remainder of the system

The practical importance of the distinction between existent adaptations and potential adaptabilities for evolutionary theory is that much of the discussion of this topic centres around the appearance of anatomical structures which show little or no capacity to vary in the lifetime of the individual of which they are a part It is perhaps unfortunate that our methods of observation make it much easier to examine the static and anatomical adaptations than the perhaps more fundamental physiological adaptabilities But so it is

It is important to realise that in considering evolution we have to deal with changes which take place on a number of different time scales, and that we may wish to discuss adaptations and adaptabilities at each of these levels We can for instance, conceive of a physiological adaptability which is developed as a result of an epigenetic adaptability which itself has been evolved as a consequence of a genetic adaptability An example might be an estuarine form, which could survive great and rapid fluctuations in the salinity of the water But this physiological adaptability might only reach a high efficiency in animals which from their earliest stages have developed in estuarine conditions And

finally, the capacity to respond to a fluctuating situation during growth by the development of an efficient physiological mechanism of this kind might itself be the result of an evolutionary process. Similar distinctions can be made in respect to anatomical adaptations. The development of thickened skin on the hands of the manual worker is an example of a developmental adaptation. The capacity to produce such an adaptation may be brought into being, or at least enhanced, by evolution, and in that case would be a result of evolutionary adaptability

It may be as well to summarise, with the aid of a diagram (Fig 23), the forms in which the adaptability of organisms may be manifested, and to compare them with the formulation given by Sommerhoff In the interests of simplicity, consider an organism which develops into only two components, *A* and *B*, which then interact together to produce the final adult condition, *P*. Adaptability consists in the ability to maintain *P* relatively constant even when environmental influences impinging on the organism alter some component of it (which we will take to be *A*). The entity which is buffered in this way is, in the last analysis, the fitness, but in complex organisms there may well be buffering of some earlier component which influences fitness.

The diagram in Fig 23 summarises a number of different conditions We may first suppose that the organism develops in some 'normal' environment and produces the components *A* and B Then let some unusual environmental condition supervene (arrow 1) which shifts *A* to *A'*, in an adaptable organism, *B* will concurrently alter to *B'*, the change being of such a kind that *A'* and B' interact to produce P' which is quite near P. Here the environmental change (arrow 1) corresponds to the environmental variable Et_1 in Sommerhoff's definition of directive correlation, the final state (or fitness) *P* to his Gt_2, the whole responding organism to his Rt_1 The 'coenetic variable' CVt_0 is not directly represented in our scheme, it is the factor which causes *B* to change in relation to the alteration of *A* in such a way that *P* is kept constant. We cannot yet state, for organisms in general, what this factor is All one can say, of a general kind, is that its operation can be improved by natural selection, and

it may be that it is brought into existence by the same agency

It is, however, almost certainly not possible to consider it as an earlier value of the environment, as Sommerhoff does in his definition of adaptation If the abnormal environment operates at a somewhat earlier stage in development (arrow 2), it may

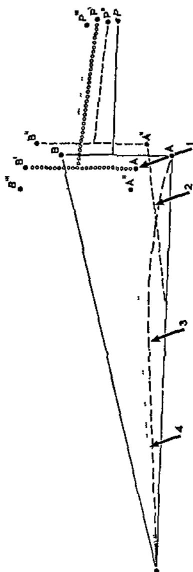


FIGURE 23

Adaptation and acclimatisation The organism is represented as developing, from left to right of the diagram, *into two components A and B, which react to give a final state P* Environmental influences, acting either only in the adult stage or throughout development, are indicated by the heavy arrows

produce an additional effect, usually known as acclimatisation. That is to say, if the organism is subjected to the environment over a period, the response of A is likely to be modified, so that it shifts only to A'', the response of B, correlated with this, is also modified to B'', and A'' and B'', interacting with each other, will produce an end-state P'' which is in many cases nearer to P than P' was.

In both the circumstances so far described, in which the abnormal environment acts either for a short duration (arrow 1) or a somewhat longer one (arrows 1 and 2), the adaptation, if any, occurs in a period which is small in comparison with the whole lifetime. Such processes can be referred to as 'direct' or 'physiological' adaptation, either simple, or, in the second instance, facilitated by acclimatisation. They are normally reversible. When they are, they operate to preserve physiological homeostasis.

We need also to consider what may happen when the special environment acts for a longer period, which makes up a large part or the whole, of the life-span (arrows 1, 2, 3 and 4). It may be that the character which in the standard environment would have developed to A now appears as A'''. This character is a typical 'environmentally produced modification' or 'acquired character'. It may or may not be of a kind which is advantageous to the organism. We can speak of the process as 'developmental adaptation' in those cases in which the new value A''', interacting with the B component (which may be unaltered or may have changed to B''') produces a fitness P''' which is near to the original P. An organism in which the A component can be altered in this way has 'developmental flexibility' and if the alteration preserves its fitness it has 'developmental adaptability'.

We may find, however, that even though the special environment operates continuously for a long period (arrows 1, 2, 3 and 4) the component A emerges unchanged. This is what is meant by saying that its development is canalised, or that it exhibits homeorhesis. Such a system is the contrary of one which shows developmental flexibility. It safeguards the organism against changes from A to A''' of a kind which lower fitness. On the other hand, if the development of A is canalised, and that of B is flexible, and modified by the environment, the interaction of

A and the modified *B* is bound to lead to a reduction of fitness The complete homeorhesis of *A* deprives the organism of developmental adaptability; it will lead to a lowering of fitness in a new environment, unless the development of *B* is also canalised. One might be tempted to say that the only component of the organism which can, with perfect impunity, be canalised by and for itself is the fitness, if any other component is canalised, it will be advantageous to canalise everything: (but strictly speaking this is illegitimate, since fitness is not a 'character', in the sense in which that word is normally used) In organisms which live in fluctuating environments, as they all do, natural selection has to try to find a compromise between canalisation of each separate component, and flexibility combined with correlations of a kind which result in developmental adaptability

Although one can distinguish these processes of direct adaptation, acclimatisation, developmental adaptation and canalisation, we have as yet extremely little understanding of the mechanisms by which they operate. The greater part of the discussion of adaptation which has been actively carried on since the earliest days of biology has been concerned not with the processes which that word can be used to denote, but rather with the structures and functions referred to when it is employed in its other sense

Types of adaptation

It is abundantly found in the living world that the structures and activities of an animal or plant are very precisely adapted to the environments in which it lives The nature of the processes by which this situation has been brought about during evolution provides one of the major problems for biological theory. The hypothesis of the inheritance of acquired characters suggested that in some way or other the effects of functioning become themselves inherited It has usually been interpreted to mean that the reaction between the organism and its surroundings has, as one of its results, an effect on the germ-plasm such that new hereditary changes occur, of a kind which determines the development in later generations of individuals suited to these particular conditions of life. Although this idea has recently been revived

in a rather nebulous form in the Soviet Union, it has been so completely rejected by the rest of the scientific world that it is hardly considered to be worthy of discussion in most of the important recent works on evolution The reigning modern view is that, in nature, the direction of mutational change is entirely at random, and that adaptation results solely from the natural selection of mutations which happen to give rise to individuals with suitable characteristics I want to argue that this theory is an extremist one, and that, in essaying to account for adaptations, it neglects to call to its aid the doctrines emerging in other fields of modern biology which can quite properly be combined with the conclusions of genetics in the strict sense In the discussion which follows, attention will be confined to animals, but there is no reason to doubt that similar arguments could be advanced in the botanical field

It will be advisable first to glance briefly at the phenomena which are usually referred to under the heading of adaptation, since they are apparently of several different kinds which have been distinguished from one another

There is, first, a category in which an animal living under particular circumstances, or behaving in a particular way, itself becomes modified so as to be better fitted for its special circumstances Examples of such direct adaptations are legion If muscles are continually and intensely used, they become thicker and stronger, if one kidney is removed from a mammal, the other hypertrophies, if the forelegs are absent at birth, or removed shortly afterwards, from rats or dogs, the hind-limbs become modified to suit the bi-pedal gait which the animals are forced to adopt, if skin is subjected to frequent rubbing and pressing, it thickens and becomes more horny, and one could multiply such instances almost indefinitely Since the precipitating causes of these direct adaptations are to be sought in the external environment they may be called 'exogenous' The capacity to produce exogenous adaptations is what we have earlier (p 144) referred to as adaptability

Secondly, there is a category of what may be called pseudo-exogenous adaptations, in which the animal exhibits characteristics similar to effects which can be called forth as direct exogenous

adaptations, but which on investigation are shown to be hereditary, and independent of any particular environmental influence We shall consider some examples of such adaptation in more detail later, since they pose one of the most striking problems to be solved

Finally, there is a very large class of *adaptations, which, as* Medawar (1951) has recently emphasised, are of quite a different nature to those of the previous category They are characterised by the fact that the adaptive feature *is of a kind which one cannot* imagine as having ever been produced in direct response to the environmental conditions or mode of life of the animal To give *two examples only,* Medawar mentions the modifications of certain epidermal cells to secrete sweat, and the development, from another part of the skin, of a transparent area which forms the cornea of the eye It is, as he says, impossible to see how any attempt to peer through an area of opaque skin could tend to cause it to become transparent The adaptation of the cornea to vision can hardly have arisen in any causal dependence on external factors, and we might therefore give the name of endogenous adaptations to this category

It is in connection with this third type of adaptation that we can as yet make the least progress beyond the current hypothesis which is content to rely on the chance occurrence of suitable mutations But even here there is a little more to be said In some endogenous adaptations the usefulness of the character *is in* connection with factors in the outside world Another of Medawar's examples, the possession of horns which serve the purposes of aggression or defence, will suffice as an instance But the transparency of the cornea is adaptive because it is suited to the functioning of another internal part of the organism, namely the retina, sensitive to the light which the cornea allows to enter Now, I think that we shall often find that the various parts concerned in such internal endogenous adaptations are involved with one another not only during their functioning in the adult animal, *but during* their development in the embryo This is certainly true of the cornea, which can be induced from normal epidermis if an eye-cup is transplanted under it at an early enough stage All

the various parts of the eye, which can function efficiently only if they have the correct relations with one another, are interdependent during their development It was shown many years ago by Ross Harrison (1929) that if the large lens of the axolotl *Amblystoma tigrinum* is grafted over the eye-cup of the smaller *A punctatum*, the lens does not grow to its full size, while the eye-cup provided with the larger lens attains a greater size than usual (Fig 24) Similarly, if the eye-cup of *A tigrinum* is transplanted

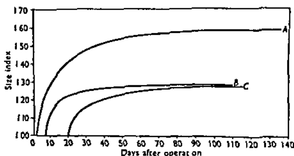


FIGURE 24

Transplantation of lens ectoderm from Amblystoma tigrinum *to* A punctatum Curve *A* growth of the tigrinum lens when associated with the eye-cup of the same species, expressed as the ratio of the size of tigrinum lens to size of punctatum lens Curve *B* similar curve for tigrinum lens associated with punctatum eye-cup Curve *C* growth of punctatum eye-cup associated with tigrinum lens, expressed in terms of its size when associated with the punctatum lens Note how in the interspecific combination the lens grows more slowly and the eye-cup faster than they would normally do (After Harrison)

under the early embryonic skin of *A punctatum* it induces a lens which is originally of *A punctatum* size and therefore relatively too small, but, as growth proceeds, the lens grows faster and the eye-cup more slowly than usual, so that they gradually achieve the normal relative proportions We might say that the internal adaptation of the lens to its retina, although endogenous in the sense that it arises within the animal, is nevertheless affected by factors from outside the lens itself, namely by the retina. The

problem it presents is therefore not wholly different from that offered by adaptations to external factors Moreover, the adaptation is in part a direct response to the influence of the retina, and is thus similar to the first category distinguished above, but there is clearly also some inherent tendency for the *A tigrinum* material to grow faster than the *A punctatum*, and in this respect we are reminded of the second category, of pseudo-exogenous adaptations

It is relevant in this connection to note that a single gene mutation may, in some cases, affect the whole structure of a complex organ whose parts have to interact together during their physiological functioning For example, genes causing polydactyly in mammals usually alter not only the number of skeletal digits, but also, and in a more or less appropriate manner, the muscles by which the digits are moved Thus when we find that the giant panda and the mole have each evolved a six-digited forelimb, and that the extra digit is provided with muscles 'adapted' to it (cf Wood-Jones 1953) we are not forced to suppose that the alterations of the skeleton and the muscles were two quite separate and disconnected events The mutation which was the first step in the evolution of the polydactyly probably altered both components the change in the muscles being, perhaps, secondary to that of the digits, on which they may be epigenetically dependent The internal adaptation of the two components is not dissimilar in principle to an exogenous adaptation, since the bones act, in effect, as the environment of the muscles (and vice versa) A remarkable example has been described by Gruneberg (1953, cf Fig 25) It should perhaps be remarked that not all genes affect an organ in such an overall manner Bithorax in *Drosophila*, for instance, converts the metathoracic integument into a mesothorax, but it does not simultaneously produce the appropriate mesothoracic muscles (Shatoury 1956, cf Fig 26) Genes of the former kind, which act on the individuation field of an organ as a whole, will be much easier to utilise in evolution than those which alter one or a few components only

We may now turn to consider adaptations towards the external environment, and firstly the direct adaptations which we have classed as exogenous, in which an animal, during its development,

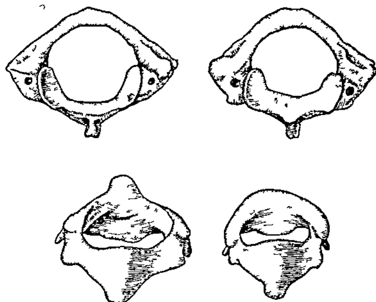


FIGURE 25

A well integrated gene-controlled modification The first two cervical vertebrae (*atlas* above and *axis* below) of a normal mouse on left and of Danfurth's short-tail' on right In the latter, the projection on the axis (the odontoid process or dens epistrophei), is absent This normally functions as a peg around which the head rotates when it is turned from side to side, as in the conventional gesture indicating 'No' In the mutant mouse, in which, as part of a general reduction in the vertebrae the process is lacking, a new

becomes modified by external factors in such a way as to increase its efficiency in dealing with them An organism which is submitted to an unusual environment during its ontogeny may, of course, become altered either in a favourable or an unfavourable manner It is a matter of first principles that the manner of its response will be subject to genetic control, whence it follows that it will be subject to natural selection. This point was well appreciated in the early days of genetics It is for instance, discussed at

some length by Baur (*1930, p 391 et seq*, a reference which I owe to the kindness of Dr. R. Snow), but little attention has been paid to it in most of the recent works on the genetical theory of evolution Natural selection will act so as to suppress or reduce the liability of the organism to deleterious modification by the environment, and at the same time to strengthen its capacities for

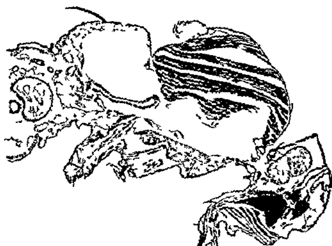


FIGURE 26
Longitudinal section of a Drosophila *adult* which exhibited a high-grade bithorax phenotype Note the absence of flight muscles in the secondary thorax (After Shatoury 1956)

adaptive responses The first type of selection leads to the evolution of narrow canalisation, the second to a canalisation which is loose with regard to certain particular types of modification, namely those which are advantageous

We have discussed earlier (p 134) the conflict between these two types of selection Narrow canalisation will be of value in rendering the organism relatively independent of effects of environmental fluctuations, but it cannot proceed too far without depriving it of the capacity to modify its development in an adaptive manner The prime selective advantage attaches to the preservation of fitness, which amounts to the homeostasis of

physiological functioning. In order to preserve this under abnormal environmental conditions, some degree of developmental modification may be necessary For instance, if newt larvae are kept in water poor in oxygen, their gills become larger and are provided with thinner walls, so that the rate at which oxygen is taken up is increased, while at the same time, the need for oxygen is reduced by a slowing of growth and reduction in activity (Drastich 1925, Figs 27 and 28), or again mangrove plants grown

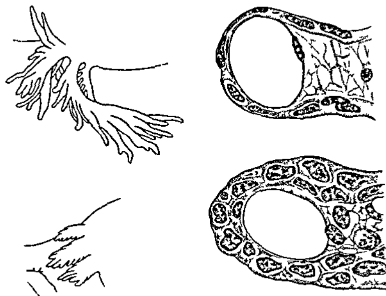


FIGURE 27
Above, left, drawing of gills of a Salamander larva kept in low oxygen tension, on the right a section through a gill filament Below, the same, from a larva kept under pure oxygen (After Drastich 1925)

in salty water develop a thick cuticle and narrowed stomata which guard them against too rapid water-loss (Baur 1930). We have then in these cases a lack of developmental canalisation, or rather a loosening of it to permit certain particular modifications, which allows the organism to achieve something approaching a

homeostasis of its maintenance-functioning This is, in fact the form normally taken by exogenous adaptations, which can be regarded as resulting from a renunciation of developmental canalisation in order to achieve physiological homeostasis

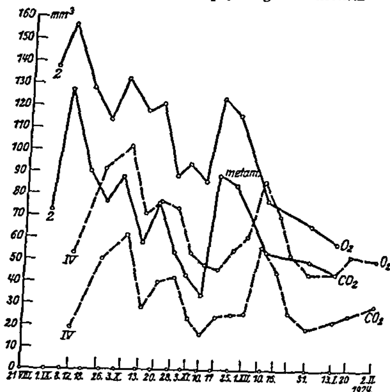


FIGURE 28
Values of oxygen uptake and CO_2 output per gramme body weight, in Salamander larvae kept under pure oxygen (full lines, labelled 2) and under reduced oxygen tension (dashed lines, labelled IV) From Drastich 1925)

A mere failure to evolve a narrow canalisation would not suffice to ensure that physiological homeostasis would be achieved Evolution has a much more ticklish task—to tighten canalisation against deleterious changes and loosen it in favour of adaptive ones Is it plausible to credit natural selection with the power to do this effectively? In the first place, it must be granted that the

precision with which canalisation is controlled by the genotype is very considerable If a stock, for instance of *Drosophila*, is selected for ability to respond developmentally in a particular way to a certain environmental stimulus, this capacity will increase, but it by no means follows that the stock simultaneously becomes more sensitive to other stimuli The response to selection is not necessarily a mere general smoothing out of the canalisation (Waddington 1957) Moreover, if one applies one and the same stimulus to a mixed population, it may produce different modifications in different individuals, and by selection from these, stocks may be built up each of which responds to the stimulus in its own characteristic manner (Bateman 1956) This suggests that it is at least not wholly unreasonable to attribute to natural selection the responsibility for evolving, from the wide range of rather precisely acting genes which seem to be available, the specific capacities for direct exogenous adaptive change which are such a usual feature of living things Yet the phenomenon is so widespread, and some of the adaptations, such as those of the newt larvae and mangrove plants mentioned above, so difficult to attribute to any well understood epigenetic mechanism, that one is tempted to feel that there may be something more behind the scenes Is there some general principle operating in all these very diverse situations? Might it be for instance, that the reproduction of enzymes is closely linked with their functioning, and that this tends to result directly, before selection acts, in developmental modifications which can be utilised for adaptive ends?

It is the remaining category of adaptations, the pseudo-exogenous, which has provoked the most discussion. We are confronted here by phenomena for which an explanation could so easily be found in a direct effect of some environmental factor, were it not that further study demonstrates unequivocally that the structure concerned is determined by the heredity of the organism, and is relatively independent of the environment The question arises whether we can bring ourselves to believe that the part which the environment can play in mimicking the condition is really irrelevant, and that the evolution of this particular adaptation has resulted from the selection of chance mutations which

might have appeared and produced the phenotypes even if the environmental effects had never existed

Some concrete examples will make the problem clearer One of the most familiar is that of the thickened skin on the soles of our feet This thickening is obviously an adaptation to the stresses which this region of the body has to bear, but, as Darwin pointed out, and as Semon (1913) discussed in a full-length paper, the thickening already appears in the embryo, before the foot has ever borne any weight The structure therefore cannot be a direct response to external pressure, but must be produced by the hereditary constitution independently of the specific external influence to which it is an adaptation The situation is even more striking when similar thickenings are found on less conventional parts of the body For instance, the ostrich squats down in such a way that the under surface of the body comes into contact with the ground at its two ends, fore and aft In just these places a considerable callosity develops in the skin (Fig 29) and Duerden (1920) showed that these thickenings make their appearance in the embryo before hatching The same thing is true of callosities which appear on the wrists of the forelegs of the African wart-hog which while feeding has a peculiar stance which involves resting on these points (Leche 1902) Again, digging animals such as the mole, are genetically endowed with heavy shoulder girdles and powerful forelimb musculature, of the type which would tend to be developed during their lifetime by their manner of life. The seal, as an embryo, develops a curvature of the cervical region of the spinal column which makes it easy for it to hold its head while swimming in the position which other non-aquatic carnivores try to attain when they are in the water and have to keep their eyes and nostrils above the surface (Wood-Jones 1953)

In plants examples of pseudo-exogenous adaptations are perhaps even more widespread and striking than in animals In [illegible]

as having adaptive va[illegible]

effects of high winds and heavy precipitation Lowland plants



FIGURE 29
View of the ventral surface of an ostrich, to show the callosities
(After Duerden 1920)

grown from seed in the hills tend to be shorter than normal, by a direct adaptation; and the opposite is true of mountain forms grown at lower levels. But neither local race becomes modified the whole way; the upland forms are genetically shorter in growth, the lowland ones taller Their adaptations are only partly exogenous, but to a considerable extent pseudo-exogenous Such races are the 'eco-types' of Turesson (1922, 1930).

The existence of such partially or completely genetically determined pseudo-exogenous adaptations has for a long time caused surprise As a recent American author has put it, 'the "best" value of the controlling factor (the morphology of the adult which controls physiological functioning) is independent of the disturbing factor (the environment)! Such genetic fixation is comparable to being sewn into one's winter underwear' (Warburton 1955). It is not quite clear from the context of these sentences whether the exclamation mark is intended to signify disbelief or mere astonishment But, surprising though they may be, the facts can hardly be denied, and it is for them that an explanation must be sought. One possibility that will be suggested is that if one lives in the Arctic it may indeed be preferable to be sewn into one's winter underwear than to risk having it blown away by an unusual gust of wind!

The genetic assimilation of adaptive characters

Many authors have argued that truly exogenous adaptations are produced by selection for the capacity to respond developmentally to the environment in an appropriate way. Baur has already been cited to this effect A much earlier author, Douglas Spalding, to whom Haldane has recently drawn attention, had thought along similar lines fifty years before In a lecture given in 1872 (see Haldane 1954) he made the following amusing suggestion: 'Suppose a Robinson Crusoe to take, soon after his landing, a couple of parrots, and to teach them to say in very good English, "How do you do, sir?"—*that the young of these birds* are also taught by Mr. Crusoe and their parents to say, "How do you do, sir?"—and that Mr Crusoe, having little else to do, sets

to work to prove the doctrine of Inherited Association by direct experiment He continues his teaching, and every year breeds from the birds of the last and previous years that say "How do you do, sir?" most frequently and with the best accent After a sufficient number of generations his young parrots, continually hearing their parents and a hundred other birds saying "How do you do, sir?" begin to repeat these words so soon that an experiment is needed to decide whether it is by instinct or imitation, and perhaps it is part of both Eventually, however, the instinct is established And though now Mr Crusoe dies, and leaves no record of his work, the instinct will not die, not for a long time at least, and if the parrots themselves have acquired a taste for good English the best speakers will be sexually selected, and the instinct will certainly endure to astonish and perplex mankind, though in truth we may as well wonder at the crowing of the cock or the song of the skylark."*

Neither Spalding nor Baur, however, find any good reason why selection should have gone so much further than would appear to be necessary, and genetically fixed a character which, it might seem, could have been directly produced in each generation They suppose that such an evolutionary step must have required very many generations, but mere lengthening of the time does not really help In its simplest form the problem is this We have a stock of animals *P* living in environment *A* If some of them are caused to develop in environment *B*, they become modified so as to be adapted to their new situation, we might call these P_b animals, and their progeny if brought back in *A*, will develop exactly like the original *P* stock But if a local race evolves in environment *B* it will become of a type *P b*, which, ideally, are indistinguishable from P_b except for the fact that if their progeny develop in *A* they will not be identical with *P* but will show some traces of the P_b characteristics What agent has operated to cause this genetic fixation of the P_b features?

In my opinion, the solution of this problem can only be found in the context of developmental canalisation, an idea which was

* For a modern discussion of animal behaviour in relation to evolutionary processes of the kind with which we are here concerned, see Spurway (1955)

not known to the earlier authors The phenomenon of pseudo-exogenous adaptation is esentially one in which the genetic constitution imposes a limitation on the degree to which the phenotype responds to environmental change The race adapted to environment *B* fails to become completely altered back to the original form when taken back to environment *A* Its development has been to some extent *canalised towards the phenotype appropriate to B*

The question is, how has this canalisation been brought about? There are several possibilities In the first place, a gene mutation may have occurred, by chance, which determines a canalised development suitable for *B* This is the hypothesis known as 'organic selection', or the 'Baldwin effect' It was advanced by Baldwin, Lloyd Morgan and others in the early years of the century Simpson (1953), who recently devoted an article to a discussion of it, describes the Baldwin Effect as taking place in three stages, which put very shortly are (1) Individual organisms interact with the environment in such a way as to produce non-hereditary adaptations, (2) Genetic factors producing similar traits occur in the population, (3) These factors increase in frequency under natural selection The gap in the argument is *between steps* (1) and (2) *Is there supposed to be any connection* between the developmental adaptations and the genes with similar effect, and if so, what? Simpson says that either there is no *particular connection, in which case the theory signifies very* little, or the connection must be by way of a neo-Lamarckian causal connection Huxley (1942) seems to put the point originally *made by Baldwin and Lloyd Morgan* more clearly when he writes that the adaptive modifications operate 'by holding the strain in an environment where mutations tending in the same *direction* will be selected' Thus according to both Simpson and Huxley, the theory of 'organic selection' still leaves the actual nature of the adaptive changes produced to the operation of *either* random mutations or Lamarckism

A similar reliance on random mutations, occurring independently of the environmental stimulus, is implicit in the work which was carried out by a group of Russian authors between

1936 and 1944 Gause, who was one of the most important of them, has described these activities in a very interesting paper, published in English in 1947 He points out that the Baldwin-Morgan idea of organic selection, appearing at a time when Mendelism had only just been rediscovered, and when most biologists believed in the inheritance of acquired characters, rapidly fell into oblivion The same principle was, it seems, independently arrived at in Russia by Lukin in 1936, and Gause and his fellow workers carried out a number of experiments inspired by it, which were published in 1941 and later

The organisms studied were *Paramecium* and *Euplotes* If vegetatively propagated clones of these Protozoa are cultured at different temperatures or in media of different salinities, any changes in shape or other properties that occur must be direct exogenous adaptations, since all the organisms will have the same genetic constitution and no natural selection can occur If, however, a culture is started from th

operate Gause showed that in several cases natural selection in a particular environment operates to produce changes, in characters such as body size, which are similar to the direct adaptations to that environment exhibited by vegetatively propagated clones This he considers to be 'organic selection' in the sense of Baldwin, since it involves the selection of genes which act in the same direction as the environment But he does not seem to conceive of the genes as controlling the response of the organism to the external circumstances, he phrases his description always as though the action of the gene was quite independent of the environment In fact, when the abnormal environment is so extreme that the initial vegetative clone cannot survive in it, although some segregating ex-conjugants succeed in doing so, he denies that this is also organic selection, writing 'With increase in salinity of the medium to 7 per cent, adaptive modifications in *Euplotes vannus* are no longer formed and various strains entirely die out Among ex-conjugants direct natural selection of viable individuals occurs In this case there is clearly no organic selection' Thus he

does not consider that the segregated genotypes which are favoured by natural selection operate by making possible an adaptation of some kind to the environment, he seems to think of *their phenotypic effect* as merely *selected* by the environment, but not otherwise modified by it In this he is probably giving to the phrase organic selection, though in modern Mendelian terms, precisely the significance which Baldwin and Morgan intended it to bear, and although he does not discuss the problem of why an adapted race, when replaced in its original environment does not always return completely to its initial form, the general tenor of his thought would undoubtedly lead him to a reliance on 'random mutations'.

The whole train of thought found in Gause, and implicit in the old idea of organic selection, is based on the over-simplification of forgetting that the environment is one of the determinants of the phenotype It is more realistic to envisage these phenomena as the selection, not of genes whose effects, though not modified by the environment, happen to be parallel to the direct adaptations, but rather of factors which control the capacity for response to the environment In these terms, we can go further in finding an explanation for partial irreversibility of adaptation It has become a commonplace that natural selection for any character will alter the general 'genetic background' of the population If the selection is for the capacity to respond adaptively to the environment, it will mould the epigenetic landscape into a new form, in which this response is facilitated and perhaps adjusted so as to reach the most favourable end-result. There will then be two ways in which genetic fixation of the originally acquired adaptive character may take place Either a gene-mutation occurs which suffices to direct development into the channel which has been prepared for it, or the remodelling of the epigenetic landscape goes so far that what was initially the side-valley, reached over a threshold, becomes the most easy path of change, so that one cannot point to any particular genes as being responsible for switching development into it (Fig 30)

The more thorough the remodelling of the landscape, the more likely will it be that some random gene-mutation will be able to

take over the switching function of the original environmental

may still ask why, even if such a mutation occurs, should it be

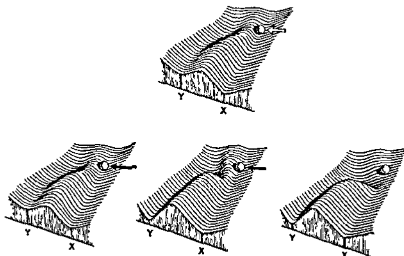


FIGURE 30

'Organic selection' (the Baldwin effect) and genetic assimilation The diagram above shows part of an epigenetic landscape, with a main valley leading to the adult character *X* and a side branch leading to *Y*, the developing tissue does not get into the *Y* path unless an environmental stimulus (hollow arrow) pushes it over the threshold The three diagrams below show ways in which the 'acquired character' *Y* might become incorporated into the genotype On the left, the original environmental stimulus is replaced by a mutant allele (dark arrow) which happens to turn up, this is 'organic selection' On the right are two modes of 'genetic assimilation' In the central one, the threshold protecting the wild type is lowered to some extent, but there is an identifiable major gene which helps push the developing tissues into the *Y* path On the right, the genotype as a whole causes the threshold to disappear and there is no identifiable 'switch gene' Note that in both the genetic assimilation diagrams there has been a 'tuning' of the acquired character, i e the *Y* valley is deepened and its end-point shifted from Y to Y'.

selected? Provided natural selection so improves the capacity to respond to the environment that the adaptive change is regularly performed, is there any advantage in going further, so that the adaptation becomes genetically fixed and occurs even in the absence of the stimulus? The answer, I think, must be an appeal to the same type of consideration which we have to call upon to explain the evolution of canalisation in general The almost universal occurrence of developmental buffering, and the generality of some degree of genetic fixation of adaptations, suggests that it is an advantage if individuals tend to produce the modal adaptive phenotype even if their particular life-history has involved circumstances which were somewhat unlike those which are most common for their fellows, and to which the adaptation is adjusted If, for instance, there was no canalisation of the growth habit of a plant ecotype, every cold spring would convert the lowland forms into alpine types unable to take advantage of a succeeding warm summer It looks as though it must be too difficult for natural selection to produce organisms which always respond in a perfectly adjusted adaptive manner to fluctuating environmental circumstances, and that *faute de mieux* it tends to fix, by canalisation, a type which is reasonably well adapted to the situation it will most frequently encounter When this occurs in a population living in an environment which remains relatively unchanged for considerable periods, it is the process which we have called canalising selection When it happens to a sub-population which is carrying out exogenous adaptation to a new environment, it converts this into a pseudo-exogenous adaptation, and the 'acquired character' becomes genetically assimilated

Since the concept of the epigenetic landscape is not well known, and since some people find it difficult to follow an argument expressed in terms of a three-dimensional diagram, it may be well to discuss these ideas in another way Let us consider first a population of animals living in some environment *A*, in which some important character, subject to natural selection, takes values within the range *a* (Fig 31) Suppose that the character is influenced by the environment The genotypes composing it will react each according to its appropriate sensitivity, and if,

in a later generation, the conditions change from those of A to those of B, some individuals may be supposed to react according to the line P, others say to Q, to give only two examples Now if the optimum for the character in environment B falls within the range b, Q will be favoured by selection but P rejected On return to A, however, a population of Q's would again exhibit the character within the range a, and no assimilation towards the modified b range would have occurred The simplest possible

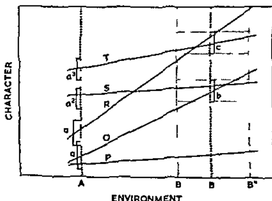


FIGURE 31
The assimilation of a character which varies in a quantitative manner

type of genetic assimilation would occur if selection favoured the building up of genotypes which produced the character within the range b even though the environment might vary from B' to B'' This would lead to the appearance of individuals which reacted more like S, and a population of these, on return to A, would show the character within the range a^2 In this case, the rejection of P in favour of Q is an example of 'normalising selection' (p 72), and the formation of genotypes which react like S rather than like Q is what we have called canalisation, while the almost complete retention of the modified b range when the population returns to A is the phenomenon which leads one to speak of assimilation

It is also possible for normalisation in B to take place around some optimum value which lies outside the range which can be

produced by direct adaptations such as are indicated by the lines *P* and *Q*. *It might be, for instance, that the optimum fell in the range c.* Then if we had purely normalising selection, with no canalisation, *the result might be genotypes which react like R, in* which the 'strength' of the genotypic tendency for the character, *and the sensitivity to the environment, have both been increased* On return to *A*, they would regress towards the range *a*, but would not quite reach it, coming say to a^1 If, in similar circumstances, canalising selection also operated, we might get genotypes reacting like *T*, which would regress only to a^3.

In Fig 31, an individual of the initial population whose reaction to environmental change is indicated by a rather steeply sloping line, such as Q, would be exhibiting only very loose canalisation It is worth considering the situation when the character of the original population is strongly canalised. As an extreme example, we can consider a character which is *entirely* unexpressed *in the* original population in environment *A*, and which is developed only when some threshold is exceeded, as, we will suppose, *it is* for some individuals when the population is transferred to environment *B* We may then postulate that the original *population* would react as indicated by line Q in Fig 32, but note that here

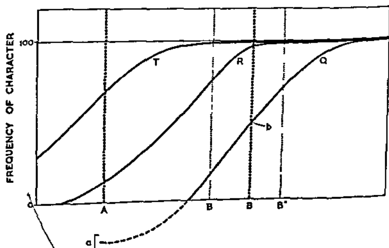


FIGURE 32
The assimilation of a quantitative character which involves a threshold

the ordinate is the *frequency* with which the threshold is exceeded and development is diverted out of the canalised normal path into the abnormal path If the abnormal phenotype has an advantage in environment *B*, there will be normalising selection to increase the frequency with which it occurs, so that the population will be changed into one which reacts like R

This may already show some assimilation, in the sense that the abnormal phenotype may appear, perhaps only in a low percentage, when a return is made to environment *A* Further assimilation could occur if canalising selection becomes important It could do so in two rather different ways In the first place, it might be advantageous to produce the abnormal phenotype in high frequency even if the environment varied throughout the range *B′* to *B″* We should then build up a population reacting like the line *T*, and this corresponds in its mode of formation to the population also indicated by *T* in Fig 31 But there might also be another form of canalising selection We have so far considered the abnormal character as unvariable, but there might be some optimum degree of expression of it in environment *B*, and if there were, canalising selection would tend to build up genotypes which produce this grade of expression throughout the *B′* to *B″* range This possibility cannot be indicated in Fig 32 which deals with the frequency of the abnormal phenotype, but not with its degree or kind of expre

course of the side valley and a shift in the end point which it finally reaches

An evolutionary process which results in the genetic assimilation of a character which was originally produced as a direct adaptive response to the environment must, in fact, be envisaged as a somewhat complex set of changes, which may proceed in sequence, or, more probably, concurrently One phase in the process is the appearance of genotypes which direct development into the 'adaptive' path, or something like it, without requiring the assistance of any special environmental stimulus This may sometimes be accomplished by quite straightforward progressive

selection of genes of appropriate activities This would suffice when the epigenetic systems in the initial population possessed a canalised system in which the adaptive phenotype could be elicited merely by pushing development out of its normal path Assimilation would then require no more than that the gene-dosage was raised above the threshold which protects the initially normal developmental path (i e it would only be necessary to *steer development over the saddle in Fig 30*) Often, however assimilation would require something more, namely the building up of a new canalised path In its simplest form this involves the selection of genotypes with only restricted responsiveness to the environment (e g those like *S* in Fig 31) In such cases, not only progressive, but also canalising selection would be operative Finally, canalising selection would, in many if not most cases in Nature, have still another task to perform, namely to guide the new path of development so that it reaches exactly the most valuable end We might call this the 'tuning' of the adaptive phenotype

It will be argued later (p 188) that this process of tuning is from the theoretical point of view, perhaps the most important aspect of genetic assimilation So far, however, most of the experimental work which has been devoted to exploring these possibilities has been devoted to the other aspects of assimilation, and has touched only incidentally on the tuning of the phenotypes

These experiments were made with *Drosophila* Very strong environmental stimuli were used, which pushed development over well-marked thresholds into quite definitely abnormal channels In a first series of experiments (Waddington 1954) pupae aged about 21 to 23 hours were subjected to a temperature of 40° C for four hours In the foundation stock, a number of aberrations in the wings were produced One of these, a breaking or even complete absence of the posterior crossvein, was selected for study Selection was applied for (and also against) the capacity to react to the environment in this manner, the 'upward' selected stock being carried on by breeding in every generation from flies in which the crossvein was broken, while the 'downward' selected were bred by taking in every generation those which

failed to respond It was immediately apparent that, as might be expected, the capacity to respond was under genetic control and became strengthened (or weakened, as the case might be) as the experiment proceeded The important point then emerged that genetic assimilation began to occur After about 14 generations, flies of the upward selected stock were found to produce a small number of offspring which developed broken crossveins even when they were not given the temperature treatment In order to speed up the further progress of assimilation, these flies were bred from and selected in normal temperatures, when stocks were rapidly produced which had a broken or absent crossvein in a high *percentage of individuals*

Similar experiments have been made by G Bateman (1956), using a number of different foundation stocks (Fig 33), and in

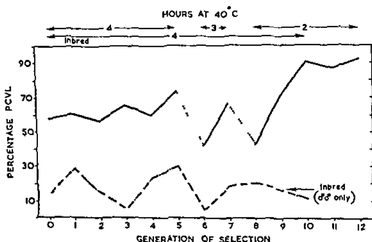


FIGURE 33

Sele t on f [illegible]
as indicated at the top, in each generation, only flies exhibiting the modification were bred from The lower dot-dash graph shows the lack of response to selection of an inbred strain (derived from the same original stock), the inbreds were treated for four hours throughout the experiment (From Bateman 1956)

some cases selecting for different environmental responses. Perhaps the most extravagant character which has been assimilated in this way is the well-known bithorax phenotype, in which the metathoracic imaginal buds develop into an accessory mesothorax. This effect is not only produced by the bithorax gene, but can be called forth by various environmental stimuli (ether vapour, high temperatures) applied to the egg shortly after laying (Gloor 1947, Maas 1948). In an experiment in which ether was used as the inducing agent, the character eventually became assimilated after some 28 generations of selection (Waddington 1956). The resulting flies are highly bizarre (Fig. 34). If such a change occurred in



FIGURE 34

A fly of the 'assimilated bithorax' stock, with the normal wings removed to show the metathorax transformed into a very complete secondary mesothorax. (From Waddington 1956*a*.)

Nature, it would certainly rank as a 'macro-evolutionary' step It seems doubtful whether in any other selection experiment such a large change has been produced so rapidly from a normal wild type stock

These experiments are, of course, artificial in the first place because the responses to the environmental stimuli are not actually of any natural selective advantage, but are only treated as such by the experimenter This, however, does not seem likely to affect the general principle of the process It is rather difficult to think of an experimental material which would be convenient for breeding through a large number of generations and which also shows clear-cut environmental adaptations of real value to the organism Attempts are now being made to study the lengthening of the posterior tracheal horns in *Drosophila* larvae kept in a liquid medium, and it seems possible that the haemoglobin formation induced in certain small crustacea by low oxygen tension might also be favourable experimental material

Another point to note about the experiments is that the later stages of the selection were based on the assumption that any tendency towards assimilation can be treated as favourable That is to

is that, *if* genetic fixation of an ecotype is valuable, this mechanism can bring it about

There are several other interesting points about these experiments. We may ask what is the genetic constitution of the assimilated stocks Is their epigenetic landscape like that in the centre of Fig 30, with a modified genetic background but also one relatively important switch gene, or is it like that on the right, in which there is no particular gene which can be singled out as more important than any other for the switch? In the first assimilated crossveinless stock which was made, there was little evidence for the existence of any definite switch gene In assimilated bithorax, on the other hand, there is a gene (or genes) on the *X* chromosome with rather strong effect This factor is recessive, and acts by 'maternal effect', causing females homozygous for it to

lay eggs which produce a moderate proportion of bithorax phenotypes It could be regarded as a switching mechanism, but it only succeeds in giving a really high percentage of bithorax individuals *if the rest of the genotype* determines a suitable epigenetic landscape with a low threshold for this modification, and some bithoraces are formed even without it

The process of selection for the ability of an animal to react adaptively to its environment, leading to the genetic assimilation of the adaptive character, provides us with a way of understanding how 'acquired' characters may become inherited without our having to suppose that the external conditions have been responsible for calling into being the necessary genetic determinants There is no *a priori* theoretical reason which would prevent us imagining that all the genes which eventually make up the assimilated genotype were already present in the population before the selection began, and only required bringing together Equally, however, it must be admitted that this supposition does not necessarily follow from any of the facts which have yet been related Most previous discussions of acquired characters and their possible inheritance have turned around the question whether the environment can provoke the appearance of new hereditary variation of an appropriate (1 e adaptive) kind It seems to be the opinion of nearly all recent authors, with the exception of Lysenko and his followers in Russia, that the lack of conclusive

evidence for the conversion of acquired characters into inherited ones which has been provided by the experiments on genetic assimilation make it necessary to discuss the matter again, specifically *in connection with them*

If one looks at the facts concerning the assimilation experiments with an open mind, one will find in several cases rather strong indications that the genetic basis for the assimilated genotype was in fact present in the initial population, while there is little that positively suggests—and that not very compellingly—that the environmental stress has called forth the new variation In the

case of the crossveinless phenocopy, the evidence for the pre-existence of the genetic variation is indeed convincing In the population from which Waddington's experiments were started, no crossveinless flies were found before the experiment began, but the number of individuals examined was not very large Bateman (1956), who repeated the work using a different stock, checked over very many more of her initial population and found a low percentage (<1 per cent) of spontaneous, naturally occurring low grade crossveinless individuals (Fig 35) Selecting

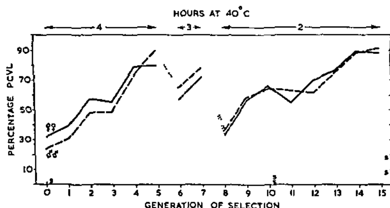


FIGURE 35

Selection for liability to heat induced crossveinlessness In this stock (Edinburgh) unlike that shown in Fig 33, there were a very few spontaneously crossveinless flies in the population from which selection started The frequency of these is shown by the points marked *S*, their numbers increase as the selection for heat-sensitivity proceeds (From Bateman 1956)

these, she was able to build up a crossveinless stock, and when this was tested against the assimilated crossveinless stock eventually derived after the temperature treatment, they appeared to be identical in genetic constitution It seems certain, then, that all the genes which enter into the genotype of the assimilated race were already present in the initial population, though in such low frequencies that it was only in a very few individuals that sufficient of them occurred together to give a crossveinless phenotype

What the environmental treatment has done in this case is to reveal sub-threshold concentrations of these genes, and thus made it possible for selection to get a hold

Attempts to carry out genetic assimilation starting from inbred lines have remained quite unsuccessful (Fig 33) This provides further evidence that the process depends on the utilisation of genetic variability present in the foundation stock with which the experiment begins

In most of the other phenocopies studied by Bateman no individuals of the abnormal type could be found in the initial populations This does not, of course, prevent us supposing that the necessary genes were present, if the assimilated genotypes involve the co-operation of large numbers of genes, and if each of these was present only in low frequency before the experiment began, it would require a search through an unmanageably large number of the starting population before an abnormal individual was found We are only confronted with a positive suggestion that new variation has arisen if it appears that the assimilated race contains a gene which we cannot imagine to have been concealed in the stock with which we began. There are actually three cases which suggest something of the kind

One of these arose during Bateman's selection for a dumpy-like phenocopy provoked by a temperature shock to the pupa An assimilated stock was eventually produced, and it was shown that an important element in its genotype was a gene with a rather strong tendency to produce dumpy wings It is probably an allele of the well-known dumpy locus Although when this gene is crossed out of the assimilated genotype into a wild-type background it causes dumpy wings to appear only in a low percentage, it seems most unlikely that it could have been concealed in the initial population, it is more probable that it has arisen by mutation during the course of the experiment The same conclusion applies even more forcibly to a dominant (with recessive lethal effect) which appeared during the selection of the *bithorax* phenocopy and caused a slight enlargement of the halteres This gene, which seemed to be identical with the previously known bx^D, actually appeared twice, once in each of the two selection

experiments which were being carried out simultaneously, but there is always the possibility that the second appearance was due to contamination by a fly which had escaped from the stock of the mutant which had by that time been established The gene did not produce a full assimilation of the phenotype, of the kind which the experiment was attempting to produce, and it was therefore eliminated from the line which was carried on and which eventually gave rise to the assimilated stock described and figured above Its importance in the present connection is that even when transferred into a wild-type background the gene gave a well-marked effect, and it certainly was not present in the original population, it must have arisen by mutation

The third suspicious case also occurred in the bithorax experiments In the final assimilated bithorax stock, an important element in the genotype is a sex-linked recessive condition which produces a 'maternal effect', that is, the homozygous mothers lay eggs which tend to develop into bithorax phenotypes When *X* chromosomes containing this genetic condition are transferred into a wild-type background the homozygous females still produce some, though rather few, bithorax offspring It is quite certain that this did not happen in the original population. The only way to escape the conclusion that the genetic determinant has arisen by mutation during the experiment is to suppose that the maternal effect is caused by two or more linked genes in the *X* chromosome, in which case the frequency of the complex in the initial population might have been so small that it would be overlooked This is, however, rather a forced explanation

We have then one case—bx^D—which seems certainly to have arisen by mutation, and two others—the dumpy allele and the bithorax maternal effect—which have probably done so Must we then suppose that the environmental stimuli have directly caused these mutations? We are forced to do so only if it can be shown that chance alone could not have been responsible for the result Now these experiments have involved very large numbers of flies The total numbers inspected are not very exactly known, but in the bithorax experiments there must have been some 200,000, and one would probably not be overestimating if one

guessed that in the whole work something of the order of half a million flies have been looked at more or less closely Is it reasonable to suppose that the observed new mutations, producing phenotypic changes of the kind for which a watch was being kept, arose by chance in that number? It is difficult to say Personally, I should not like to take the opposite view, and claim that it was unreasonable to attribute the mutations to chance I see in these facts no reason which compels one, or even very strongly urges one, to seek any other cause for the mutations which have occurred

I should not like it to be thought that I adopt this view merely to avoid the necessity of providing an explanation for an effect of the environment on mutation Recent developments have made it somewhat easier to envisage mechanisms by which such effects might conceivably operate In particular, it has been found that in many micro-organisms, such as yeasts and bacteria the presence of some unusual substrate may provoke the formation of an appropriate enzyme, and there are some reports of the synthesis of such 'adaptive enzymes' also in the cells of higher organisms The mechanism of the process is still obscure, but its reality seems beyond question Now genes can be considered to be enzymes, or at least to be in many ways similar to the more typical cellular enzymes Although it is not clear how far this analogy should be pushed, one would not, perhaps, be too much astonished if it should be found that some metabolic 'opportunity' available in the cell might tend to induce a gene mutation to an allele which in some way fitted it

A diagram which expresses the formal structure of this train of speculation is given in Fig 36 In the upper half, we have three genes, each of which produces a primary gene-product (shaded) The cell also contains materials which are dependent on the environment (triangles) Under the conditions indicated the products of genes *A* and *B* combine, together with the environmental materials, into a complex, but the product of gene *C* does not fit easily into it In the lower part of the figure, the environmental materials are shown in an altered shape The products of *A* and *B* now fit together in a different way, which leaves a

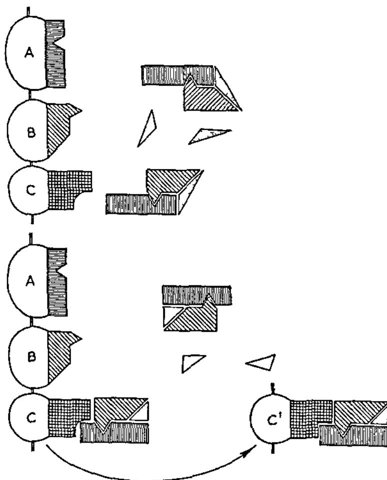


FIGURE 36

A speculation about environmentally-induced gene mutation The upper diagram represents three genes *A*, *B* and *C*, which give off a replica of a part of themselves as active gene-products In the presence of a certain environmentally produced substance (trianles), the products of *A* and *B* combine as shown. In the lower half, another environmentally-produced substance is present (triangles of different shape) The products of *A* and *B* now combine to form acomplex, which is nearly but not quite comple- mentary to *C* Then it is conceivable *that C might be caused to mutate into the fully complementary form C'*, perhaps by a mechanism similar to that responsible for the appearance of an adaptive enzyme.

position into which the *C* product could become attached provided it underwent the slight change which is indicated as the conversion of its re-entrant semicircle into a right angle The occurrence of adaptive enzyme formation makes it appear not impossible that the existence of such a potential site might stimulate the appearance of an allele to fit it

This fantasia on the well-worn theme of imaginary keys fitting into hypothetical locks is not, of course, offered in the hope of convincing anyone that such processes occur It is intended only to suggest that it may be unsafe to consider that the occurrence of directed mutation related to the environment can be ruled out of court *a priori* A mechanism anything like that suggested may perhaps seem very improbable There is also another improbability, implicit in the fact that the gene mutation, if it is to be inherited, must occur in the germ cells rather than in the somatic tissues on which the environment impinges directly (but it is

But even if improbable, such processes would not be theoretically inexplicable It must be for experiment to decide whether they happen or not So far there seems to be no definite evidence that they do Work with chemical mutagens has been held to show that some substances tend to stimulate more mutations in some genes than in others (e g Demerec 1953 and Glover 1956), but there is no suggestion that the new alleles are in any way appropriate to the metabolic potentialities of the cell It looks as though the different mutations depend on the types of active groups which ... offered for ... has only a slight effect in provoking ... which *can exploit it*, it will be difficult to detect in the face of the possibility that the gene mutation has occurred by chance But it seems wisest to keep an open mind on the subject

In the context of evolution, however, the question is probably irrelevant Natural populations can be assumed to be so *large* that any mutation of which the genotype is capable is at least potentially present The meaningful question is not whether a

mutation, considered as a change in a nucleo-protein, has occurred before or not, but rather whether the evolutionarily important phenotype is already available. There is no doubt that a phenotype such as the assimilated bithorax, if it occurs at all in wild populations, does so at such low frequencies as to be out of the control of direct selection.

There are many adaptive phenotypes which one can hardly suppose to have been present in the ancestral population before it was exposed to the environment to which the adaptation is related. It is difficult, for instance, to imagine that any ancestral ostriches had callosities just in the appropriate places and no others, before being subjected to the particular relevant environmental stress. Again, many adaptations depend on correlated changes in several characters which there is no reason to suspect would spontaneously be connected with one another in development. Canalising selection for an optimum adaptive response of the organism to its environment would tend to build up epigenetic systems producing such special phenotypes. The type of change involved is that indicated in the diagrams of Fig. 30 by the alteration of *Y* into *Y'*.

There has been less direct experimental study of this kind of adjustment of the end results of development, but there is already some evidence about it. For instance, when a temperature shock of a given intensity and duration is applied to a number of pupae of a definite age from a population of *Drosophila*, there is usually considerable variation in the type of response which is exhibited (Fig. 37). Some flies develop with a broken cross-vein of the kind discussed on p. 172. In others it may be the anterior cross-vein which is broken. Extra cross-veins may be formed in one or other of several different places, or the shape of the wing may be altered. We may say that the environmental stimulus has pushed development over a threshold and out of its normal course, but that there is no very definite alternative canalised valley along which it will then run. Now by regarding a broken posterior cross-vein as having adaptive value and selecting for it we succeed in causing this character to become assimilated, as was pointed out above, while the other abnormal types of development become

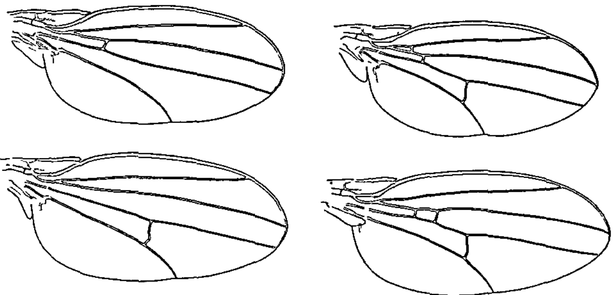


FIGURE 37
Types of wings produced by heat-shocks to Drosophila pupae Left, above, posterior crossveinless; below, anterior crossveinless, right, two types of extra veins. (After Bateman 1956)

relatively reduced, or at least, not equivalently increased in frequency If, however, selection is practised for one of the other characters, such as an extra anterior cross-vein, that character may also become assimilated and the broken posterior cross-veins remain infrequent, as Bateman (1956) has shown In these experiments selection for a particular type of environmental response has, in effect, canalised one or other developmental pathway out of the possibilities which are available in the initial mixed population

There can be little doubt that it would be possible through selection to control still more precisely the exact nature of the end result of development For instance, Timofeeff-Ressovsky (1931) has described a gene in *Drosophila funebris* which produces a break in the posterior cross-vein very similar to that with which the experiments on genetic assimilation were performed Starting with a mixed population in which this gene was present he was able by selection to build up three stocks In one of these the rest of the genotype acted in such a way that the gene usually caused a break at the anterior end of the cross-vein, in the second stock the break was usually at the posterior end, while in the third it occurred at either end with equal frequency A similarly precise control over an assimilated phenotype has not yet been realised It would presumably take longer to do than to bring about the mere assimilation of a crossveinless type, regardless of whereabouts in the cross-vein the break occurred. But there seems every reason to suppose that if a break at the anterior end was advantageous while one at the posterior end was harmful selection pressure could assimilate the former to the exclusion of the latter

It might sometimes be advantageous in the artificial selection of crops or livestock to utilise processes similar to those which have just been discussed That is to say, one might apply some environmental stress for the specific purpose of forcing into expression genetic factors which, although present in the population, remain imperceptible under normal circumstances and therefore inaccessible to selective pressures When such a procedure is consciously adopted, one might refer to it as 'forced selection' But it must be recognised that no clear line can be drawn between forced selection under environmental stress and a hypothetical

normal selection under no stress, since the second of these situations cannot, strictly speaking, ever arise In all populations under any circumstances, the environment plays some part in determining the phenotypes It is not simply that the variations in the environment contribute to the variance in the phenotypes, but the modal environment systematically influences the modal phenotype, and this influence would remain even if the inter-individual environmental variance could be reduced to nil All selection which impinges on the phenotype is a selection of the capacity of a genotype *to respond to an environment*

The phenomena of genetic assimilation in higher organisms are very closely related to problems which have been extensively studied by micro-biologists, particularly in connection with the evolution of drug-resistant strains of bacteria The extensive literature can be approached through the volumes edited by Gale and Davies (1953) and Sevag and others (1955) It has frequently been found that when a strain of bacteria sensitive to some deleterious drug are grown in the presence of the substance a resistant strain eventually emerges A considerable controversy has occurred between those who hold that the 'adaptive' hereditary character was present in some few cells of the original strain before contact with the drug and those who argue that a hereditary alteration was a consequence of this contact It seems now to have been demonstrated with some certainty that, in some cases at least, the former alternative is correct, since from a sensitive strain it is possible, by suitable and highly ingenious methods, to isolate resistant clones without ever bringing the cells into contact with the drug (Lederberg and Lederberg 1952, Cavalli-Sforza and Lederberg 1956) This is true not only for strains which owe their resistance to single genes of large effect, but even for those in which the resistance depends on the synergistic action of many factors These results do not, of course, in any way exclude the possibility that the actual defences of the bacterium against the drug may depend on a process of adaptation (which, owing to the short lifetime of a bacterium, might be regarded equally well as a developmental or a physiological phenomenon) But there remains little reason to suggest that the

hereditary characteristics of resistant strains have been brought into being by induced adaptive mutations

The ultimate basis of the phenomenon of resistance in bacteria is therefore very similar to that to which genetic assimilation in higher organisms has been attributed Both processes are founded on the selection of pre-existing mutations rather than on mutations induced by the conditions which are adapted to There are, however, considerable differences between the developmental and genetic systems of bacteria and higher organisms, and these condition the manner in which the selection processes operate in the two cases In higher organisms we are usually dealing with characters (e g the shape of an organ) whose epigenetic basis is much more complex than a drug-resistance is likely to be, we are almost always confronted with systems of genes rather than single genes To compensate for this, one might say, selection can take advantage of the greater genetic flexibility of the sexual mechanism of recombination. And finally, in the cases which have been studied in higher organisms, the situation mentioned at the end of the last paragraph is realised, and the epigenetic development of the character selected for is an adaptive response to the presence of the selecting environment. We thus find ourselves selecting for genetically reshuffled, complexly determined, capacities for adaptive modification, while the bacteriologists are usually dealing with relatively simply determined characters whose genetic basis is changeable in the main by mutation alone It is a measure of the efficiency of sexual reproduction as an evolutionary mechanism that exceptionally precise adaptations of very complex characters are no less possible in the relatively small populations of higher organisms than the simpler adaptations are in the much larger populations of bacteria.

EPILOGUE

THE ADJUSTMENT of the end results of development is perhaps the most generally important aspect of the mechanism which has been suggested for genetic assimilation. In this connection it acquires a much wider importance than as merely providing an explanation for the fixation of ecotypes. We have been led to conclude that natural selection for the ability to develop adaptively in relation to the environment will build up an epigenetic landscape which in its turn guides the phenotypic effects of the mutations available. In the light of this, the conventional statement that the raw materials of evolution are provided by random mutation appears hollow. The changes which occur in the nucleoproteins of the chromosomes may well be indeterminate, but the phenotypic effects of the alleles which have not yet been utilised in evolution cannot adequately be characterised as 'random'; they are conditioned by the modelling of the epigenetic landscape into a form which favours those paths of development which lead to end-states adapted to the environment.

As many have pointed out, the gravest defect of modern science as a general philosophy is its exaggerated atomism. Even before the advent of Darwinism, Tennyson in *In Memoriam* heard the voice of Sorrow:

'The stars,' she whispers, 'blindly run;'

but it was above all the doctrine of Natural Selection which placed Man himself inexorably in a sheerly mechanistic universe whose parts, although they indeed reacted on one another, were existentially quite independent. Darwin's theory was interpreted to mean that all living things, man included, had been brought into being by the collocation of two entirely independent factors, on the one hand the occurrence of mutations whose nature was totally unconnected with any ambient circumstances, and on the other a sieving process in which the environment merely selected from among organisms which were offered to it ready-made as

units of being, not in any way of potentiality Any further influence which the environment might have was degraded to the status of mere 'noise' in the system of genetic determination This was a glaring example, in another field, of that Bifurcation of Nature against which Whitehead (1929) inveighed so strongly in epistemology, and many humanist and religious authors, from the Victorians to such present-day critics as Barzun, have drawn attention to its damaging effects on man's spiritual life

We are now beginning to see in what way such an outlook is scientifically inadequate, what real processes it leaves out of consideration, and why the simplifications it makes are unjustifiable According to the view I have been developing, organism and environment are not two separate things, each having its character in its own right which come together with as little essential inter-relation as a sieve and a shovelful of pebbles thrown on to it The fundamental characteristics of the organism—its Form, to use the term which was employed in the Introduction—are time-extended properties, which can be envisaged as a set of alternative pathways of development, each to some degree, greater or lesser, a creode towards which the epigenetic processes exhibit homeorhesis And in this way we can conceive of organic Form, not only as occupying four dimensions instead of only [illegible] ties as well as what is actually [illegible] the epigenetic landscape, with [illegible] with steep or gently rising sides, with cols and hanging valleys of more or less well defined contours, provides a rather crude but in some ways serviceable way of visualising the possible ways in which the developing system can be modified Natural selection, whose direct operations impinge on the phenotypes which result from the interaction of genotype and environment, favours systems of genes which respond to the local situation by producing well adapted organisms It thus does something more than merely favour organisms which are, for some reason or another, fitter than their fellows, it builds into the epigenetic system tendencies to be easily modifiable in ways which are adaptive And since animals, at least, have some ability to choose their environment, natural selection has still

another dimension in which to work towards a fitting of the two together—towards that appearance of End, which was the other of the major problems of biology with which this discussion began.

The integration between the living organism and the rest of the world is, admittedly, by no means complete The characteristics built into the epigenetic landscape influence, but do not completely determine, the phenotypic effects of mutations, and processes of genetic drift may go some way to loosen the bonds between the animal and its surroundings But we can now see that these elements of discontinuity are set within a framework of essential connectedness

APPENDIX

SOME PHYSICO-CHEMICAL ASPECTS OF BIOLOGICAL ORGANISATION

H KACSER

INTRODUCTION

THE BELIEF that a living organism is 'nothing more' than a collection of substances, albeit a very complex collection of very complex substances, is as widespread as it is difficult to substantiate The demonstration that the organism is a sort of container within which chemical and physical processes take place, or that parts of the organism or part-processes obey the laws which have been found to apply to the inanimate world, established, of course, only a prima facie case What is required is a demonstration that those properties and types of behaviour which we believe to be

result of the complex interplay of inanimate substances and processes The problem is therefore the investigation of *systems*, i e components related or organised in a specific way The properties of a system are in fact 'more' than (or different from) the sum of the properties of its components, a fact often overlooked in zealous attempts to demonstrate 'additivity' of certain phenomena It is with these 'systemic properties' that we shall be mainly concerned. These only arise as a result of a particular type of organisation, i e particular functional relationships between the components, and these properties, of course, 'disappear' when that organisation is destroyed or altered This is the reason why the physical sciences, often dealing with the same components as those in organisms, in many cases fail to say something of relevance to the biologist There are no concepts in chemistry or physics equivalent to genes, regulation, epigenesis,

pleiotropy, phenocopy, acquired character, etc , precisely because *these* are properties *only* possible in systems of greater complexity than have been subjected to detailed analysis by these sciences

The first task, therefore, is to show that systems, composed of 'orthodox' inanimate components, can display properties which we normally associate with living organisms In so doing we cannot hope to describe a single system showing all or many of such properties, partly because of the limitation of space and time and partly because of the limitation of mathematical theory We shall therefore have to describe a number of systems each displaying particular aspects of biological organisation A system which displays, say, multiple effects of a single substitution will be associated with the concept of pleiotropy, but an organism showing *this* will have additional properties very different from *those* of the system Unavoidably, we shall fail to show relationships which arise from inter-system interactions when we extrapolate to the system as a whole, but as a first step there is no other way.

The interest in discussing systems of this kind is that it may *throw light on the mechanisms and processes* underlying biological phenomena The great achievement of genetics has been the *demonstration that certain portions* of the chromosome control to a large extent the development and behaviour of organisms Although we believe the elaborate circumstantial evidence that the genes act in this fashion, it is desirable that, like Justice, they should *manifestly* appear to act This activity is primarily revealed in the single chemical and physical steps, which are the 'atoms' of the organism To demonstrate the genes in action then, requires the language of the molecular calculus instead of the Mendelian arithmetic which revealed their presence

The appropriate method of description is that of chemical kinetics The relevance of any conclusions will therefore depend on how far the assumptions of kinetics may be assumed to apply to living organisms It is immediately obvious that, strictly speaking these assumptions never apply However, the predictive

...sions

between the 'real' and 'ideal' situation This is for example the case in the biochemical mutants of micro-organisms when they [illegible]

assume that the kinetic treatment is applicable The justification for this however must in each case be that its consequences are experimentally demonstrable In fact whenever a particular kinetic model is equated with a genetic or physiological phenomenon that should not be taken as 'proof but merely as a possible mechanism The model should be regarded as a working hypothesis which can be verified or disproved by performing appropriate experiments Since it would be tedious to reiterate these reservations every time they will from now on be assumed to be constantly borne in mind by the reader

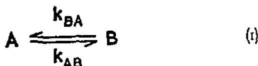
A further difficulty must be mentioned To explain phenomena which are described in concepts of one branch of science in concepts of another involves semantic and epistemological questions of some importance As these problems are outside the scope of this work they will only be dealt with when they impinge directly on a particular example How far this neglect may invalidate the arguments must be discussed in another place

CATALYSIS IN CLOSED AND OPEN SYSTEMS

Closed systems

The most commonly examined systems in kinetics are closed ones i e systems in which no matter enters or leaves Under any given set of conditions they will approach a time-invariant state known as thermodynamic equilibrium Open systems on the other hand are those which have an inflow and outflow of matter Certain of these will also reach a time-invariant state which is known as the steady state There are important differences between the two and we shall examine both

The simplest closed system is composed of two species which interconvert monomolecularly



The rate equations for each component may be written as

$$\frac{dA}{dt} = k_{BA}B - k_{AB}A$$

$$\frac{dB}{dt} = k_{AB}A - k_{BA}B.$$

At equilibrium both rates are zero and we obtain the well known solution

$$\frac{\bar{A}}{\bar{B}} = \frac{k_{BA}}{k_{AB}} = K$$

where $\bar{A}$ and $\bar{B}$ are the equilibrium concentrations. In order to show the behaviour of the system as it changes towards the equilibrium state, however, we must consider it as starting at a certain initial state and solve the rate equations If A_0 and B_0 are the initial concentrations of A and B respectively and x the amount changed at any time, then

$$\frac{dx}{dt} = k_{AB}(A_0 - x) - k_{BA}(B_0 + x)$$

On integration this gives

$$x = \hat{x}[1 - e^{-(k_{AB}+k_{BA})t}]$$

and

$$\hat{x} = \frac{A_0 - KB_0}{K+1}$$

Since

$$A = A_0 - x$$

and

$$B = B_0 + x$$

the rate equations for A and B are

$$A = \frac{K(A_0+B_0)}{K+1} + \frac{A_0-KB_0}{K+1} e^{-(k_{AB}+k_{BA})t} \tag{2}$$

$$B = \frac{A_0+B_0}{K+1} - \frac{A_0-KB_0}{K+1} e^{-(k_{AB}+k_{BA})t} \tag{3}$$

For $t = \infty$ we find

$$\bar{A} = \frac{K(A_0+B_0)}{K+1} \tag{4}$$

$$\bar{B} = \frac{A_0+B_0}{K+1} \tag{5}$$

and of course

$$\frac{\bar{A}}{\bar{B}} = K \tag{6}$$

We can also represent the results diagrammatically by curves A

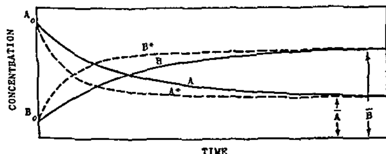


FIG 1

and B We find that the ratio $\bar{A}/\bar{B}$ is independent of the absolute value of the rate constants and the initial concentration but that the values of $\bar{A}$ and $\bar{B}$, although independent of the rate constants, depend on the values of A_0 and B_0 In fact, the sum $(A_0 + B_0)$, i.e the total initial concentration of substances, determines the values of the final concentrations The rate equations contain in their

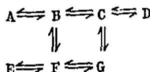
exponential term the sum of the rate constants This means that the *absolute values* of these constants (not merely their ratio K) determines the *rate* at which equilibrium is approached

A catalyst acts by increasing the rate of reaction in such a way that the forward and backward rate constants are increased proportionately, so that the ratio of the new constants is the same

$$\frac{k^*_{BA}}{k^*_{AB}} = K \tag{7}$$

although $k^*_{BA} > k_{BA}$ and $k^*_{AB} > k_{AB}$
The addition of a catalyst to the system or change in catalytic concentration, will therefore leave the values of $\bar{A}$ and $\bar{B}$, and their ratio $\bar{A}/\bar{B}$ unaffected but will affect the rate of approach *This is indicated by the second set of curves in Fig 1*

No matter how complicated the closed system is, e g



the same general conclusions hold

rate constants Whether any, some, or all of the steps in the system are catalysed, or what the quantity or efficiency of the catalysts may be, cannot affect the equilibrium properties of the system which depend only on the ratio of the rate constants i e the equilibrium constants The rate of change of the system, however is a function of the rate constants as well as the initial distribution, and the behaviour of the developing system can therefore be influenced by alterations of these parameters *This is summed up in* Table 1

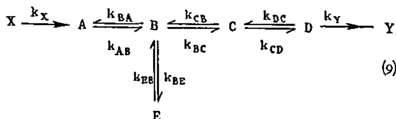
TABLE 1 CLOSED SYSTEM

$$\left.\begin{aligned} \bar{N} &= f(K, No) \\ \bar{N}/\bar{M} &= f(K) \\ dN/dt &= f(K, No\ k) \end{aligned}\right\} \tag{8}$$

We have described these well known relations in some detail because they differ in important respects from the relations of open systems Although for certain purposes an organism (or part thereof) may be considered as a closed system there are serious limitations to such a view This is evident when we consider the fact that there is catalytic (enzymatic) control not only of development but of phenotype, a phenomenon contrary to the invariance of the equilibrium composition with respect to changes in the values of the rate constants This is not so in open systems which we shall now discuss

Open systems

Consider the system



which is 'open' by virtue of the fact that it is constantly being supplied by X and constantly passes out Y It is assumed that the source X is so large in quantity that the amount converted into A makes no difference to its concentration or that its concentration is maintained constant by some other (external) mechanism It will also be noted that both k_X and k_Y are irreversible steps This is not the only type of open system* and we shall deal with others later, but it is one which reveals a number of pertinent properties In the above system (which assumes constant volume) the components X, A, etc, may stand for concentrations or for masses, and the equations and deductions apply to either formulation This is not necessarily so in other systems which may be discussed.

* See Burton, *J Cell Comp Phys* 14 (1939) 327 Denbigh Hicks and Page, *Trans Faraday Soc* 44 (1947) 470 Bertalanffy, *Science* 111 (1950) 23

The rate equations for the components in the system are

$$\frac{dA}{dt} = k_X X - k_{AB}A + k_{BA}B$$

$$\frac{dB}{dt} = k_{AB}A - (k_{BA} + k_{BC} + k_{BE})B + k_{CB}C + k_{EB}E$$

$$\frac{dC}{dt} = k_{BC}B - (k_{CB} + k_{CD})C + k_{DC}D$$

$$\frac{dD}{dt} = k_{CD}D - (k_{DC} + k_Y)D$$

$$\frac{dE}{dt} = k_{BE}B - k_{EB}E$$

The system will reach a time-invariant composition when the amount flo

condition i

concerned

pointed out that while the equilibrium in closed systems is the state of maximum entropy, the steady state is one of minimum entropy production owing to the flux of matter through it On equating all rates to zero and solving we find the following values for the steady state concentrations of the components If the product Xk_X, which is the constant amount flowing into the system, is written Q_X we have

$$\left.\begin{aligned}
\bar{A} &= Q_X\left(\frac{K_{AB}K_{BC}K_{CD}}{k_Y} + \frac{K_{AB}K_{BC}}{k_{CD}} + \frac{K_{AB}}{k_{BC}} + \frac{1}{k_{AB}}\right) \\
\bar{B} &= Q_X\left(\frac{K_{BC}K_{CD}}{k_Y} + \frac{K_{BC}}{k_{CD}} + \frac{1}{k_{BC}}\right) \\
\bar{C} &= Q_X\left(\frac{K_{CD}}{k_Y} + \frac{1}{k_{CD}}\right) \\
\bar{D} &= Q_X\frac{1}{k_Y} \\
\bar{E} &= Q_X K_{EB}\left(\frac{K_{BC}K_{CD}}{k_Y} + \frac{K_{BC}}{k_{CD}} + \frac{1}{k_{BC}}\right) \\
&= K_{EB}\bar{B}
\end{aligned}\right\} \quad (10)$$

where the capital K's are the equilibrium constants and the lower-case k's the rate constants

The first and most interesting aspect of the s s (steady state) values is that rate constants and not only equilibrium constants enter the expressions of all of them This means that the absolute values of the rate constants, which can be altered by the presence of catalysts, determine the final values of the components This fact makes it immediately possible to control the composition of the steady state, the 'adult' state of the system, by the control of the nature and quantities of the catalysts present

Secondly, the s s values are independent of the initial concentrations since these do not enter the expressions This means that with a given catalytic situation, the system will reach the same state, both as far as its composition (ratio of components) and as far as the *absolute values of the individual components* is concerned, irrespective of the quantities and distribution in the initial state

Thirdly, the rate of supply, the flux rate enters as a factor in all of the s s values Its variation will therefore affect all components in the same way and it will be eliminated when the ratio of two or more components is considered This means that, although the absolute size of the adult system will depend on the flux rate, its composition, and all other properties depending on the relative values of the components, will be unaffected by size of the system

The explicit solution of the rate equations for a system with more than three components is not possible (this applies equally to closed system equations) although particular cases (i e known numerical values of the constants) can be evaluated We know, however, that the solutions will be of the form

$$\begin{aligned} A &= Qx(\bar{A}x + A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} + A_3 e^{-\lambda_3 t} + \quad) \\ B &= Qx(\bar{B}x + B_1 e^{-\lambda_1 t} + B_2 e^{-\lambda_2 t} + B_3 e^{-\lambda_3 t} + \quad) \end{aligned} \qquad (11)$$

where $\bar{A}_x$ is the steady state value, as given before, divided by Q_x, i e $\bar{A}_x = \bar{A}/Q_x$ For n components in the system there will be $n - 1$ exponential terms The λ terms are the solutions of an equation of the $(n - 1)$th degree involving rate constants only The A, B, C, etc , terms are functions of equilibrium constants,

rate constants and initial concentrations Their sign may be +ve or −ve and hence these equations can show maxima and/or minima

The relations in this open system are summed up in Table II

TABLE II OPEN SYSTEM

$$\left.\begin{array}{l} \bar{N} \;\;\;= f(Q, K, k) \\ \bar{N}/\bar{M} = f(K, k) \\ dN/dt = f(K, N_o, k) \end{array}\right\} \quad (12)$$

We may now discuss some relevant biological properties in relation to the mechanisms operating in the open system Since most reactions in an organism are mediated via enzymes we shall consider the situation where all steps are catalysed

KINETIC MODELS

Regulation

Let the system start with a given complement of catalysts for all steps, and a certain initial concentration of reactants (some of which may be zero) It will proceed from this initial state towards the s s according to equations (11) and will approach the values (10) If we now take a second system with the same catalytic concentrations, but altered initial concentrations of reactants, it can be seen that the development of the system will be different but that the same *s s* values will be *approached* This property of open systems has been called 'equifinality' It is illustrated diagrammatically for one of the reactants in Fig II

If for simplicity we assume that in the second system all initial concentrations were halved, and if the final concentrations are higher than the initial ones, the 'half system' will take longer to reach the same 'degree of completion' than the first system (Both will of course reach the *s s* '*at the same time*', viz when $t = \infty$) The precise time relationships are complex and will depend on the system, but comparing 'equal stages' in most cases the 'half system' will arrive at that stage in less than twice the time

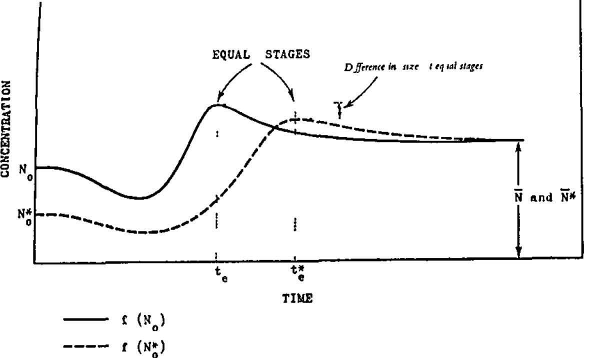


FIGURE II

Equifinality of open systems Rate of approach towards the steady state of the same component in two systems differing in initial concentrations

We can compare this behaviour with the phenomenon of regulation shown particularly in echinoderms and amphibia We may provisionally identify the cytoplasm with the reactants and the nucleus with the catalysts (In later discussion *we shall examine* the justification for this and any modification required)

The many studies involving separation of early cleavage cells or fusion of two, have shown that the amount of cytoplasm does not affect the ability to produce a normal type of organisation. In some cases of amphibia the adult appears to reach the same (or very nearly the same) absolute size The same applies to mammalian identical twins As far as time relationships are concerned few data are available, but Tyler (*Biol Bull* 68 (1935) 451) reports for example that a giant (double) sea urchin reached the pluteus stage in 64 hours while the normal took 84 hours—less than twice the time At that stage the sizes were still different and there is no eveidence in the case of sea urchins as to whether the 'abnormal plutei would develop into normal sized adults

The system described will reach the same s s even if the initial *proportions* of the substrates are altered The many experiments which have been performed using constriction, cauterisation and removal of parts of eggs have established the existence of gradients For normal development only restricted parts (usually in the middle) can be removed There is no direct analytical evidence whether these gradients are composed of substrates or enzymes In the latter case one would, of course, predict that development will be abnormal since the *catalytic* proportions are altered by experimental removal of one end of the gradient There is no evidence bearing on the behaviour of eggs where one can be sure that substrate proportions only have been altered

The flux rate Q_X *only affects the absolute values of the* s s, leaving the proportions invariant If we associate Q_X with the rate of feeding of the organism (or the rate of supply of some essential metabolite), the invariance of the composition may be compared with the general observations on starvation Within limits (about which more will be said) many organisms can show remarkable changes in size depending on the food situation But they still retain largely the same proportions (Fig III)

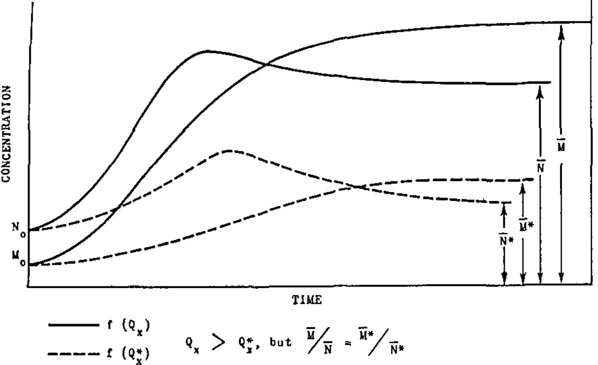


FIGURE III
Effect of flux rate on the steady state values and proportions of two components The two systems differ in the rate of entry (or conversion) of X. (Change of X or k_x or both, since $Xk_x = Q_x$)

'Regeneration'

Related to the property of equifinality is the reaction of the system to disturbances of the s s If some part of the reactants is removed, the system will return completely to the s s The removal has in fact brought it to a new 'initial' state from which it will proceed in the normal manner (Fig IV)

Numerous studies on amputation have shown a great divergence in the power of regeneration of different species or of different organs in one organism The most remarkable for their completeness are in the planarian worm and the hydroids Small portions of these can regenerate into the complete organism In some ways this is very similar to embryonic regulation, since it is not only one organ but the whole functional organisation which develops from the portion The identity of the systemic mechanism in both cases does not, of course, argue that organisms showing large powers of regulation as embryos necessarily show good regenerative abilities (This will become evident when we discuss more complex systems) But it does suggest that the two phenomena are closely related and may be influenced by the same type of agencies

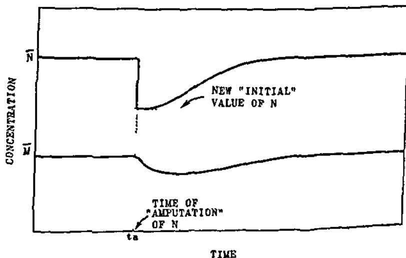


FIGURE IV

Effect of disturbance of the steady state by removal of part of one component The disturbance spreads to other components and the rate of recovery of one of these is also indicated

Temperature effects

Temperature has an effect on both the equilibrium constants K and the rate constants k The precise relationships, which are dealt with in physico-chemical textbooks, need not concern us here The rate constants are always increased with increase of temperature according to an equation of the form

$$k = Ae^{-E/RT} \tag{13}$$

The magnitude of E, the activation energy of the step, then, determines the temperature coefficient of the reaction *rate* For a reversible step the values of E for the forward and backward constant are, in general different and hence their ratio (i e $k_F/k_B = K$) at two different temperatures will be different In many cases the effect on the rate constants is much greater than that on K It should be noted that K may either increase or decrease with a rise in temperature (depending on whether it is an exo-thermic or endothermic reaction) When these facts are considered in relation to equations (10) and (11) it is clear that two systems kept at different temperatures but identical otherwise, will differ both in rate of development and s s values and proportions Even if dK/dt is small for all K's, the effect on the k's will result in a change in the s s (equations (10)), unlike the effects in a closed system

Since all rate constants may have different activation energies, only very general inferences can be drawn One may distinguish two classes of rate constants, the diffusion constants which have very low temperature coefficients, and the reaction constants which have high to very high coefficients If we assume that in system 9 the constants k_X and k_Y are diffusion constants while the others are reaction constants, the effect of a rise in temperature can be assessed as follows consider two systems S_L at a low and S_H at a high temperature Assume that for the temperature difference considered the effect on the diffusion constants k_X and k_Y is negligible, while all other rate constants are doubled in S_H Assume also that the equilibrium constants change little with temperature On inspecting equations (10) it is clear that the s s values of the components in S_H will be reduced (except D) since

$Q_X (= Xk_X)$ and k_Y remain constant while the other terms are reduced. The rate of development of the system towards the s.s. will of course increase

Numerous observations show that this temperature relation is found in many organisms Thus in *Drosophila* all stages of development can be accelerated by culturing at higher temperatures At the same time the size of the larvae and insects is smaller This relation is often used for producing large larvae for embryological purposes by low temperature culturing Similar observations have been made for *Fannia* and *Paramecium* (see the review by Gause, *Trans Connect Acad* 37 (1947), 17) The case of mammals is complicated by the thermostatic mechanism operat- [illegible]

phenotypic differences as well as changes in expressivity and penetrance may be cited However, no statements concerning the magnitude or direction of the change can be made unless the precise mechanism of a process is known

This general relation of the diffusion and reaction constants neglects the possible difference in heat loss occasioned by the difference in temperature in organisms which are not perfectly poikilothermic

'Substitutions'

We have already referred to the fact that the absolute values of the rate constants enter the expression for the s s values of the components. The influence of a change in the amount of a catalyst on the composition of the 'adult' system is of obvious genetic interest Since, however, we have not yet introduced the concept of the gene, the present section will merely describe some of the consequences of substituting one catalytic situation for another This will be identified with a change in the genetic situation, but the justification for this will be left to a later section.

Let us again compare two systems S_1 and S_2 of type (9) with s s solutions (10) Both start with the same initial concentration of reactants, are 'brought up' at the same temperature and are 'fed'

at the same rate Q_Y To fix completely the development and final state of the system we must fix the quantity of each catalyst Let these quantities be the same in both systems for all steps except one Let this step be D to Y Our two systems will, therefore, differ in the value of k_Y, the 'excretion' constant S_1 (with k_{Y1}) will reach values $\bar{A}_1$, $\bar{B}_1$, etc On making the 'substitution' k_{Y1} to k_{Y2}, we obtain the system S_2 ($\bar{A}_2$, $\bar{B}_2$, etc) It can be seen (10) that k_Y enters the expression of all components but in different ways While in $\bar{D}$ it will have its full effect, being the only constant involved, it can only affect part of the value of the others We thus see that the result of a single change in a rate constant controlled by a single catalyst can have multiple effects on the components of the system The magnitude of the effect will depend on the absolute value of the other constants involved and may be negligible for some

If we next choose the step B to C for our substitution, the situation is similar It will be remembered that a change in catalytic amount changes the values of k_{BC} and k_{CB} but leaves their ratio K_{BC} unaltered $\bar{A}$, $\bar{B}$ and $\bar{E}$ will be affected since they all contain k_{BC} $\bar{C}$ and $\bar{D}$ on the other hand will have the same values in both systems The degree of multiplicity of effects will therefore depend on the position of the affected step in the system While the situation is clear in this simple system (components to the 'right' of a step being invariant towards step substitutions), it becomes less obvious when closely interlinked part-systems are considered

'Pleiotropy'

These substitution effects are clearly in line with genetic facts and theory Firstly they show that, by varying the enzymic situation, phenotypes of varying composition, yet having the same basic organisation, can be obtained Secondly, pleiotropic effects are the normal consequence of kinetically linked reactions The effect described is 'genuine' or 'primary' pleiotropy. In more complex systems other higher level interactions will occur Whether these should be described as 'secondary', 'physiological', or 'spurious', is mainly a verbal matter, and will be determined

by the methods and/or the views of the investigator Although a 'hierarchy of causes' can be constructed from a system particularly when the main effects are spaced out in time it is clear that the mutual relations of the components are what they are *ab initio* and are operative all the time

Whether pleiotropy is detected at all will also depend on the method adopted. Thus, if the character studied (i e the particular function of the system) is for example dependent on all the components which are affected the single substitution will be found to have a single effect But the more detailed the analysis the more pleiotropy will be uncovered—until, quite suddenly, all pleiotropy disappears, namely when (or if) a measurement of the rate constants is made

Returning to the case where the single substitution difference was taken at the step *B* to *C*, we found pleiotropic effects on *A*, *B* and *E* but none on $\bar{C}$ and $\bar{D}$ From our general analysis of the rate equations (11) however, it is clear that the absolute values of k_{BC} and k_{CB} enter the rate equations for C and D, since the λ s are composite functions of all rate constants. This means that there is a pleiotropic effect on the developmental pattern of *C* and D but that it disappears as they approach the s s

A number of cases are reported where such a relation has been observed The case of siderocyte anaemia in the mouse may be cited (cf Grüneberg *The Genetics of the Mouse*, p 239) The recessive gene *f* when homozygous has two 'main' effects flexed tail and belly-spot In addition these animals show a transitory anaemia which disappears at about the second week after birth In fact, since the two 'main' characters 'may fail to manifest themselves on account of inhibitory genetic modifiers and unknown environmental conditions', while the anaemia shows "very little if any, normal overlapping", the latter appears to be a better character for genetic identification "The condition of the blood at birth may thus safely be used as an indicator whether a given animal is genotypically *f/f* or not"

This observation may have considerable generality The invariance of $\bar{C}$ and $\bar{D}$ towards substitution in *B* to *C* and *C* to *D* is a particular example of the phenomenon of buffering Since a

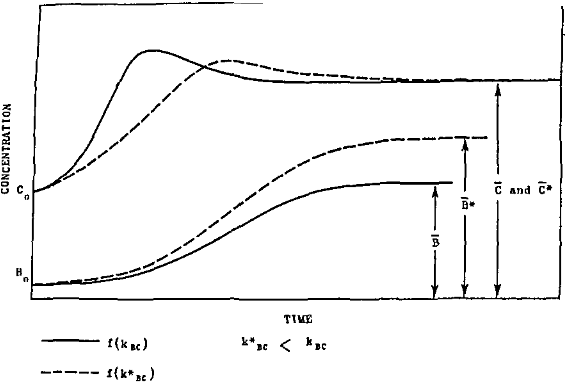


FIGURE V

Effect of substituting the catalyst (differing either in quantity or efficiency) acting *between B and C*

This affects the rate *and* final value of *B* but is only detected in the rate behaviour of *C*.

separate chapter will deal with this aspect a few remarks only will be made There are probably a large number of *pleiotropic* effects which are buffered out in the phenotype of the adult and are therefore not detected by the normal methods of scoring As in the case of *fff*, scoring of an embryonic character may be a better or safer procedure More important still there may be a large number of genes whose presence even remains undetected because all adult characters are more or less buffered to one value

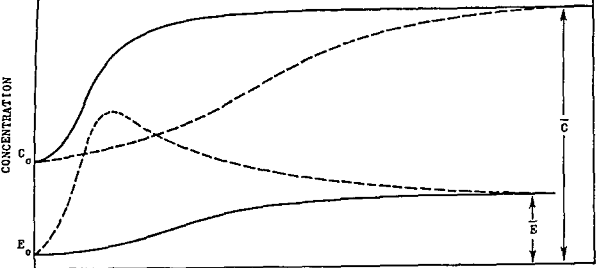
Developmental abnormalities

A mechanism showing that a substitution can have an effect *only* on the development, leaving all s s values constant, is also operative in system (9) The equation for the 'branch component' E, shows that the individual values of k_{BE} and k_{EB} as such do not enter the expression, but only their ratio K_{EB} Neither K_{EB} nor the rate constants enter the s s values of the other components The amount or nature of the catalyst for B–E, therefore, does not affect any s s values of the system On the other hand k_{EB} and k_{BE} do enter the rate equations of all components in the usual way and hence an alteration of the catalytic concentration at B–E will alter the course of development of the system but will leave the final state unaltered

We can appreciate the effect of such a change if we take the [illegible]

will be diverted into E but later this 'disturbance will disappear Fig VI shows this diagrammatically for E and another component, say C

Cases of this kind are probably very difficult to recognise unless the developmental 'abnormality' is spectacular In a segregating population this sort of variation might easily be attributed to environmental conditions It can, however, be a very important evolutionary mechanism. The effect of increasing k_{EB} and k_{BE} can be regarded as a 'time-delay', or a 'stretching of metabolic time', since it retards the approach to the s s without affecting it This may be of considerable survival value in a situation where length of development must be closely related to *for example,*



——— $f(k_{EB}, k_{BE})$, $k^*_{EB} > k_{EB}$, $\frac{k^*_{EB}}{k^*_{BE}} = \frac{k_{EB}}{k_{BE}}$

– – – $f(k^*_{EB}, k^*_{BE})$, $k^*_{BE} > k_{BE}$

FIGURE VI

Effect of substitution at the catalytic step B to E The steady state values of all components are unaltered The increase in the valuesof k_{BE} and k_{EB} produces an overshoot in E and a corresponding delay in the rate at which C reaches its final value

the plant life cycle For instance, transfer of an insect to another ecological situation may demand a longer period of pupation, and this mechanism achieves this without changing the adult phenotype.

Although no single gene has been reported which shows this phenomenon, there is ample evidence in *Drosophila* that length of development can be altered by selection without (apparently) affecting the characters of the adult

In this connection the mutant 'spheroidal' in *D funebris* (Crew and Auerbach, *Proc Roy Soc Edin* 57 (1936–37), 255) may be mentioned The mutant affects the size and shape of the eggs (as its name implies) and is therefore a 'maternal effect' However, the females carrying *sph/sph* are indistinguishable from +/+ females and again males and females hatched from spheroidal eggs are wild type in appearance Taking as our developmental cycle the period from adult mother to adult daughter we can compare the pattern of +/+ and *sph/sph* Both start and finish at the same point but their paths differ by the 'transient' appearance of the egg shape

'Larvae'

If the 'branch component', *E*, is not regarded as a single substance but a sub-system (or the first component of such a system), the behaviour discussed above describes in a formal way the passage of an organism through a larval stage The complexity of the branch system can, of course, be considerable, and the time of its duration prolonged The emergence of the final state may be gradual or may be triggered off by a threshold phenomenon The significant property is that a temporary organisation is built up, which may appear to approach a s s, but whose built-up material is eventually 'sucked back' into the main channel, to be reorganised in the form of the adult The interesting case of the axolotl shows how well adapted the branch system can become, and how difficult it is in practice to decide whether a system is near to the s s or merely near a meta-steady state

Site of gene action

Returning to the effects of a substitution at B–C we may raise the question of the 'site of primary gene action' At first the obvious answer is that it must be at *B* and *C* because this is where

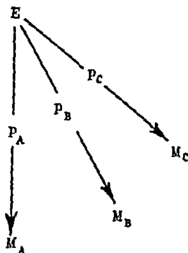
the rate constant has been changed But we have seen that $\bar{C}$ is unaffected by this. Can B then be considered as the site? Inspection of the equation for B (10) shows that k_{BC}, the affected rate constant, appears as one of three terms The contribution which this term makes to the total B may be large or small depending on the values of the other constants It is, however, precisely the same as its contribution to E Both B and E are equally affected by a change in the value of k_{BC} From the point of view of effect, there is nothing to choose between the two $\bar{A}$ will of course also be affected, but in a somewhat different way owing to the additional term $1/k_{AB}$ In more complicated systems the situation may arise where the quantitative effect of a substitution may be very slight in the 'immediate neighbourhood' of the changed rate constant, due to compensation by a buffering mechanism, but may have serious consequences in a 'distant' part It is, in fact, very difficult to assign any precise meaning to the term 'distance' with reference to these problems, except perhaps by the number of intervening components Certainly the magnitude of an experimentally determined effect is no sure guide (cf $\bar{C}$) as to the kinetic proximity of the component to the affected step

A suggestion concerning the recognition of distance has been made by Gruneberg in connection with the flexed-tail belly-spot, anaemia pleiotropy of *f/f* mice "Now it seems that whenever a gene has constant and inconstant manifestations, the constant manifestation is nearer the primary gene action than the variable one" From this it is argued that the anaemia is 'nearer' than the other manifestations

Our considerations do not lead us to such a simple relation It would only be true if each manifestation were a necessary consequence of the preceding *manifestation*

$$M_A \text{ causes } M_B \text{ causes } M_C \text{ causes } .$$

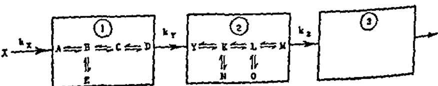
If all three manifestations are causally connected to a preceding event (and this seems a more reasonable view) the constancy of manifestation will depend on how well the respective paths P_A, P_B and P_C are buffered with respect to environmental or genetic variations The kinetic proximity of each to E is unimportant. The discussion of buffered systems will deal with this more fully



Sub-systems

So far we have considered a number of properties of systems of type 9 in relation to some well-known biological properties of living organisms However, system 9 (and more complicated versions of it) have other properties which are clearly contrary to biological facts For example it is immortal as long as it is fed by X, it does not reproduce itself, its adult properties are determined by the catalysts present as a full complement from the beginning it shows *complete* regulation and regeneration towards all temporary disturbances, and it can grow to an unlimited size dependent on the supply of material We shall therefore enquire what new aspects arise when we modify or complicate the basic organisation so far considered

System 9 consisted of five components and each was considered a recognisable character of the system If we extend our system by combining a number of sub-systems of type 9 we obtain the following system



We have seen (10) that the s s value of the last component of sub-system 1 was

$$\bar{D} = Q_X/k_Y$$

The rate of supply to sub-system 2 is

$$R_{(2)} = \frac{dD}{dt}k_Y$$

but at the s s it will be

$$\bar{R}_{(2)} = \bar{D}k_Y$$
$$= Q_X$$

The same applies to all other sub-systems arranged in series At the s s the rate of supply is Q_X to *all* sub-systems irrespective of the catalytic situation in each As far as substitutions are concerned, each sub-system is autonomous and the s s values for its components will be of the same form as in (10) Pleiotropic effects on adult characters therefore operate only within sub-systems (if they are organised in this particular way) If we take the whole sub-system (or a composite function of it) as a 'character' we can see that it is possible to effect changes in composition and absolute values of characters 1, 2, 3, etc , independently of each other This eliminates a great deal of pleiotropy that one might expect in causally dependent arrangements * The developmental path, however, is affected, but in a unidirectional way Substitutions in a sub-system affect the development of succeeding systems but not of preceding ones This is a consequence of the fact that they are connected by irreversible steps

It should be noted that a change, say in k_Y, the supply constant for sub-system 2, does not result in any changes in the s s values of that system (or any subsequent one) This invariance is achieved by an appropriate adjustment of the value of $\bar{D}$ so that

$$\bar{D} \times k_Y = Q_X = \bar{R}_{(2)} = \bar{R}_{(3)} = \ ..$$

Sub-system 1 may in this instance be regarded as a buffer for the s s values of the subsequent systems, towards changes in k_Y This

* (Cf the system described by Waddington, *J Gen* 51 (1953) 243, where genes acting on the same process are described as 'homodynamic')

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invariance is maintained in spite of the intimate causal dependence of the systems on k_Y. A cursory inspection of the scheme would not give this answer.

'Epistasis'

The s.s. value of any component of a sub-system may be altered by all the rate constants entering the expression. The quantitative relations resulting from substitutions of several constants (several 'loci') will be additive if single components are taken as characters. If, however, the character of the sub-system is a function of several components, involving ratios or products, interaction effects arise. A numerical example will make the point clear.

If we take as the character of sub-system (1) the ratio of its last two components

$$\phi = \bar{C}/\bar{D}$$

From (10) we find

$$\bar{C}/\bar{D} = K_{CD} + \frac{k_Y}{k_{CD}}$$

Assuming two 'alleles' for each of the rate constants involved we can find the values of ϕ for all four combinations. Choosing convenient magnitudes

$$K_{CD} = \tfrac{1}{2},\ k_Y = 2, \qquad k_{CD} = 2,$$
$$k^*_Y = 1; \qquad k^*_{CD} = 8;$$

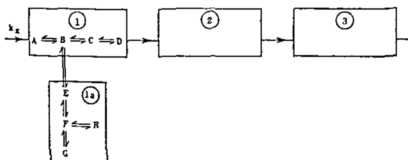
				'Expected' value	
'Allelic' combination	K_{CD}	$\frac{k_Y}{k_{CD}}$	$\bar{C}/\bar{D}$ (×100)	*Additive hypothesis*	*Factorial hypothesis*
$k_Y\ k_{CD}$	½	1	150	−50	×⅔
$k^*_Y\ k_{CD}$	½	½	100	−75	×½
$k_Y\ k^*_{CD}$	½	¼	75		
$k^*_Y\ k^*_{CD}$	½	⅛	62.5	(25)	(50)

From the differences in the first three combinations the 'expected' value for the fourth on the additive hypothesis is 25 while the

actual value is 62 5 A factorial analysis of the differences gives a value of 50 We have arbitrarily chosen the ratio $\bar{C}/\bar{D}$ as the character In more complicated systems, with feed-back or divided flows, single components can show epistatic effects, since the function of their s s values contains terms with ratios or products of rate constants

Size effects

We have already mentioned the branch component E and that it may be the first term of a sub-system The behaviour of this type of sub-system differs, however, from those described above



The s s values will be as follows

$$\bar{B} = Q_X\left[\frac{K_{BC}K_{CD}}{k_Y}+\frac{K_{BC}}{k_{CD}}+\frac{1}{k_{BC}}\right]$$

$$\bar{E} = K_{EB}\bar{B}$$

$$\bar{F} = K_{FE}K_{EB}\bar{B}$$

$$\bar{G} = K_{GF}K_{FE}K_{EB}\bar{B}$$

$$\bar{H} = K_{HF}K_{FE}K_{EB}\bar{B}$$

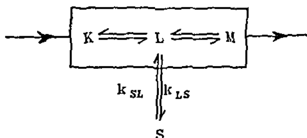
We note that no rate constants of sub-system (1*a*) (or any subsequent to (1)) enter the s s values Except for $\bar{B}$ it acts like an equilibrium system This is because at the s s there is no flux of matter *through* it, although it is of course in dynamic equilibrium with $\bar{B}$. While we can thus make no changes to the s s values by altering the sub-system rate constants, the effect of $\bar{B}$ is of interest Substitutions in (1) affect the components of (1) in a pleiotropic manner as discussed before In so far as these affect $\bar{B}$, they will

have the *same* effect on *all* components of (1*a*) i e it will be a quantitative effect only

Irreversible effects

Temporary disturbances, whether fluctuations in material supply or temperature, initial changes in quantities or 'amputations' — all affect the developmental pattern but not the s s of the systems so far considered This is so because the components of the systems were all considered soluble, each step reversible (except the inter-system steps) and all steps first order reactions While this may be true for some biological components it is clearly not true for all

The introduction of insoluble components will have the following consequences



Kinetically a solid is distinguished by the fact that its activity does not depend on the amount present The rate at which it is converted is therefore constant

$$-dS/dt = k_{SL} \times S = \text{constant}$$
$$= S_k$$

(S_k like Q_x is a rate)
The rate at which it is produced however depends in the usual way on the concentration of L

$$dS/dt = k_{LS}L$$

which will vary during development The component S will not reach a stationary value except under the very special condition when

$$k_{LS}L = S_k$$

In general L will have a different value If

$$k_{LS}L > S_k$$

the s.s. will be the one when all other components have constant value and S a constant *rate of increase*, since

$$\overline{dS/dt} = k_{SL}L - S_k$$

There are many biological properties which show nearly constant rate of production (hair and nail growth, red blood corpuscles, etc.). This is not the same as maintaining a constant quantity in a 'soluble system' where the amount present determines the amount being produced.

If

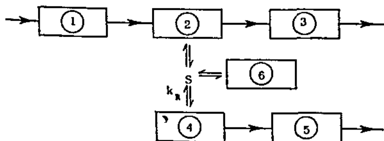
$$k_{LS}L < S_k$$

the s.s. system will contain no S, though it may be temporarily present during development.

In view of the constant activity of S the absolute quantity of S at any time is unrelated to the state of the system at that time but is dependent on the past history. Temporary fluctuations of, for example, Q_x, will be buffered out in the soluble components but will leave their mark on S.

S itself can therefore be regarded as a buffer or reservoir. A sudden temporary increase in Q_x will be smoothed out rapidly, since a large amount will spill over into S. Sudden cessation of Q_x will have less serious consequences, since S is still being supplied at the constant rate S_k. The earlier in the sequence such a reservoir is situated the more effective it will be as a buffer and the more rapidly the disturbance will be damped.

Component S may be connected to more than one sub-system,



In this situation it would act as a constant source of supply to the

same form as the open system with which we started Instead of Q_x a new flux rate is R_s substituted This is however not simply S_k, but also a function of the formation constant, k_R, and the other constants in the system

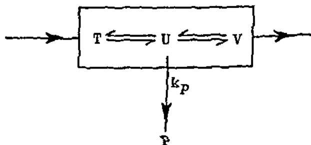
$$R_S = S_k f(k_R\ k_1, k_2, k_3 \quad k_4)$$

At the s s as before, the flux through all sub-systems from (4) onwards will be R_S, but this will now be affected by substitutions in (4) Substitutions in (5), (6), etc , will, of course, not affect R_S Epistatic effects will occur in components of (4)

We thus see that the result of interposing a solid component has the effect of making subsequent systems invariant towards fluctuations of material supply The system can now 'feed intermittently (as most higher organisms do) and yet maintain constancy of part of its organisation The buffering is achieved by an expansion or contraction of the amount of S It is complete buffering for systems (4), (5), etc , and partial for (1) (2) (3), etc

'Phenocopies'

Another type of organisation which records the effects of temporary fluctuations is the following



The component (or sub-system) P is irreversibly connected as a branch to another sub-system The rate equation for P is

$$dP/dt = k_p U$$

and hence it will vary according to the fluctuations in the developmental history of U As in the case of the solid S it will reach a stationary rate of increase and not a s s value

$$\overline{dP/dt} = k_p\bar{U}$$

Let us assume that the variation in U is such that it reaches high values during part of its history but settles down to a low s s value The plot for dP/dt may then be as follows

FIGURE VII

Comparison of the effects of a fluctuation (e g temperature) and a substitution (change in one of the k s) on the quantity of an irreversible component Note that the ordinate is the rate of production of P and that the quantity (or concentration) is given by the area underneath each curve The curves shown here result in the same increase in P by either of the altered systems The resulting total P s are identical except in their past history

The quantity of P is given by the integral i e the area underneath the curve Now compare this 'normal curve with one where the system has been subjected to a temporary fluctuation in say, temperature Let us assume this fluctuation has resulted in a

temporary larger accumulation of U The result on dP/dt is given in the second curve Although the beginning of the effect will coincide with the beginning of the fluctuation there is a lag in the return to the normal curve owing to the fact that U itself will show a lag in returning to its normal values Although both $\bar{U}$ and $\overline{dP/dt}$ will be the same in both cases, the total quantity of P will be greater for the second case The effect on P is therefore permanent The same increase in the quantity of P can be achieved at constant temperature by a suitable substitution in some rate constants This is shown in the third curve The areas under the fluctuation and substitution curves are the same and hence $P_F = P_S$ If P (or a function of it) is the character, then the two will be indistinguishable

This situation may be compared to the production of phenocopies The effect of a single substitution can be imitated by an appropriate treatment The two effects are clearly not identical particularly with respect to pleiotropic effects But these may be buffered out so that, at a superficial level of analysis at any rate the two may be said to be the same

Another interesting feature concerns the time of treatment It can be seen from Fig VII that there is a time interval during which the bulk of P will be produced Fluctuations within this period will therefore have the greatest effect while considerably before or after no significant alteration in the total quantity will be made This is in line with the observations of sensitive periods for many phenocopies

Although it was stated that P does not reach a s s value but a constant dP/dt, it is clear from Fig VII that P effectively reaches a constant value, a pseudo-s s value, since it increases only insignificantly after a certain time Components of this type are therefore laid down at more or less definite periods, as distinct from true s s components which are constantly being maintained at that level In most cases where it has been investigated it has been found that the sensitive period coincided with a physiologically critical period where some structure such as an *Anlage* is formed We may suggest that the latter peiod is in turn determined by the 'kinetically crit'cal period' of a pseudo-steady state component

'Determination'

Components like *P* which are produced 'once and for all' probably form the majority of structural components of an organism In the discussion above it was not specified whether *P* was soluble or insoluble The analysis in fact, applies to either Similarly the production of *P* was described as irreversible, but the same general conclusions can be reached if a reversible reaction is considered, so long as the forward step is very much greater than the backward The composition of such a component may therefore change slowly with time and it is a matter of experience from tracer work *that structural components have a much smaller turnover rate* than 'metabolic' components Too much emphasis is occasionally laid on the 'dynamic state of body constituents' A great number maintain their integrity over long periods of time These more permanent components will differ significantly from the 'dynamic' components and conclusions derived for one class do not necessarily apply to the other

It can be seen that the process described has an important bearing on the problem of determination While a reversible system shows complete regulation a system containing a number of permanent components cannot retrace its steps completely Thus if we picture a system with a number of components whose kinetically critical periods are spaced out in time it follows that there is an increasing inability of the system to reconstitute itself Different systems may differ considerably in the time at which such processes occur Some may have passed such periods at the time which we choose as zero and which we identify as the time of fertilisation The 'initial' state of the system has of course a history of its own Systems may differ as much in this as in their future developmental history

'Maternal effects'

In a similar way one may interpret the occasional influence of the genetic constitution of the mother on the phenotype of the offspring During oogenesis materials are being supplied to the oocytes by the maternal system Although we have seen that neither proportions nor quantities of components in the initial state influence the

s s of a reversible system, it is now clear that irreversible processes will be influenced This is not the only type of maternal effect, and another will be mentioned when we discuss the nature of the gene

BUFFERED SYSTEMS

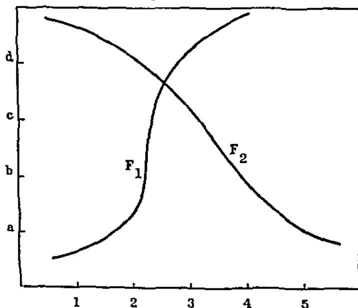
A system can be completely defined by a set of parameters and the functional relations between them These parameters may stand for concentrations, quantities, rate constants, temperature, time, distance, volume, etc Some of these may themselves be functions of other parameters not explicitly occurring in the set of equations describing the system In such a case it is either implied that, for the purpose of analysis, no change in the value of these parameters will be considered, or that, in fact, they do not change Some parameters may usefully be described as the 'external' or environmental parameters, some as 'internal' or those of the system in the restricted sense of the word In any particular instance it may be difficult to decide into which class to place a certain parameter, or whether to change its classification when different aspects of the system are being considered, but often the choice is quite clear We shall be concerned with the effect of a change in the external parameters on the system as well as the effect of internal changes

General characteristics

The statement that a parameter belongs to the system implies that it is expressed in a functional relation to at least one other In consequence most parameters are functions of many others, and a change in one will, in general, affect many others In any particular system, however, there will be some parameters which are very nearly or completely unaffected by changes in some of the others The conditions of this invariance or buffering will be the subject matter of this chapter It is desirable to state at the outset that a statement such as 'the system is buffered' has no meaning What may be significantly asserted about a system is that certain functions of its parameters are invariant with respect to certain changes A well known case may serve as an illustration Certain mixtures of acids and salts in solution known as pH buffers, will, on addition of acid or alkali, maintain the pH unaltered The function of

the system that is invariant is the H ion concentration Other functions, such as concentrations of particular molecular species, or osmotic pressure, are obviously not invariant when more acid has been added In fact changes in these functions must take place in order that the pH may remain constant Furthermore, the system may be subjected to changes other than the addition of *H* ions, such as changes in temperature or dilution Towards these the pH is not necessarily invariant And lastly, complete invariance over all possible ranges of H ion addition will not be maintained The buffer can only cope within certain limits, and even within these the buffering is *not absolute, i e dpH/dH* has a *small finite* value This function is the degree of buffering and is not necessarily linear

To describe the behaviour of a system in terms of its buffering [illegible] to state (v) The function under [illegible] which are changed, [illegible] These limitations of the term 'buffering' must be clearly borne in mind, if it is to be used in any but a metaphorical sense We may use a diagrammatic representation of the phenomenon as follows



On the ordinate are plotted the values of the variable parameter, and on the abscissa the values which a particular function in the system has under these conditions. Within a certain range of the variable the degree of buffering is given by the slope of the line, verticality being complete buffering for that range It is clear from the diagram that a knowledge of the behaviour of the two functions within a certain range, say *b–c*, is not necessarily a guide to their behaviour outside We must now enquire as to the possible mechanisms which may bring about such invariance of a function towards changes in certain parameters

The first type is in a sense a trivial one and may be called 'invariance by exclusion' It operates by the fact that the alleged change in the parameter is cancelled as far as the system is concerned Let us take, for example, a function whose value is dependent on temperature It is clear that the presence of conditions which *insulate the system against changes of temperature*, applied externally will have the effect of buffering the system with respect to such changes Thus, for example, the provision of a constant temperature by the incubation of eggs, or the surrounding of the embryo by the thermostat of the maternal body, ensures a high degree of temperature independence What has happened in these cases is that temperature as a variable has been *excluded* (or at least reduced) Functions of such systems are therefore buffered against changes in environmental temperatures because, in fact, they never experience any changes The problem of how this invariance by exclusion is brought about is another matter, and may involve other mechanisms But these do not enter the discussion if the system is chosen as the embryo only

The second type is best described as 'invariance by insignificance' Briefly it relies on large quantities—large, that is compared with the effect which the change produces We may take as an example a system which consists of two substances only, which are in equilibrium such that one of them is greatly *in excess* of the other

$$A \rightleftharpoons B, \quad A \gg B$$

Change in an external parameter, say again temperature, may

shift the equilibrium towards B, say to twice its original value This, however, will constitute only a very small reduction in the *quantity* of A, which is therefore practically invariant, while B has increased 100 per cent However, not all functions involving A are so buffered, as is evident when the ratio A/B is considered This would be reduced by a factor of nearly two A number of functions or 'characters' which are known to be dependent on the same substance may therefore show very different responses to a particular change, depending on what function of the substance is concerned in the character The insignificance phenomenon is connected with the experimental limitations of the methods of measurement, and this aspect has been described as the 'scale effect' For it is clear that on an appropriate scale even the insignificant changes can be quantitatively defined. It is, however, a fact that the accuracy of most methods of measurement is some function of the absolute values of the quantity measured If there- [illegible]

erable part in many biological processes, although its demonstration is often difficult

The third type of mechanism is probably the most common and most important, and it is the one to which the term buffering is most appropriately applied Its nature is suitably described by the term 'invariance by compensation' Unlike the first type, changes of parameters do affect the system and unlike the second their effects are not necessarily negligible Or, to be more precise, it is the initial effects which are not negligible It is the property of this mechanism that these initial effects set into operation a chain of further effects whose outcome is the eventual cancellation (or partial cancellation) of the initial effect Thus, its main characteristic is that of the feed-back An important aspect of its operation is that there is a time-lag between the onset of the change and the completion of the compensatory process The range of this time-lag extends from fractions of seconds to years, and on this basis important distinctions between 'physiological responses' and

'acquired characters' have been made These will be discussed later.

Within the class of compensatory mechanisms two sub-types may be distinguished, described *with reference to the outcome* of the process, by homostasis (*homos* = same) and homoiostasis (*homoios* = similar) respectively

The complete regeneration of parts of an organ or the restoration of the s s after a disturbance are examples of the first sub- [illegible]

change in this situation will shift this equilibrium to a new value Provided no elimination of any alleles through drift has taken place, the restoration of the original selective situation will result in the restoration of the original gene frequencies (This is often described as *genetic homeostasis* although the root *homeo* appears to be derived from *homoios* and not from *homos*)

The second sub-type is exemplified by pH buffer systems, or by the hypertrophy of one of a pair of organs if the second is amputated or destroyed The reaction of the system tends to restore some function to its original value (pH or overall functional capacity) but inevitably leaves some other aspects of the system in a different state

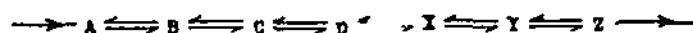
The adjustment of a system to very nearly the same state, the buffering of one parameter by adjustment of others, is probably much more frequent than the complete restoration of the status quo The *demonstration* of this, however, depends on the sensitivity of our analytical methods, and in biological material there are formidable obstacles to the complete analysis of a given case The multiple interactions in biological systems, *which make the* unravelling of causal connections so difficult, are in fact the causes of the remarkable buffering capacity so often encountered

Number and complexity

Both the time-lag and the degree of buffering will depend on a large number of factors Except for very simple systems, *it is not* practicable to develop quantitative arguments Since most of the

biological cases are associated with very complex systems, only a qualitative description of some of the factors involved will be given

In a system consisting of many members a number of factors will be important in determining its behaviour in two different circumstances The first is the actual number of members Let us take a simple sequential chain, for example,



and consider a disturbance imposed on the steady state of this system This may be, for example, the removal of part of one of the components, say *C* Now since *C* is in feed-back connection with eventually all other members, the initial effect on *C* is more quickly damped and the effect of the disturbance on its neighbours smaller than if the system consisted of, say, three members only This is so because the rate of recovery of *C* depends on the instantaneous values of its immediate neighbours, *B* and *D*, which will both decrease immediately after the disturbance This decrease will be the less the more rapid is the recovery of *B* and *D* and this again depends on *their* neighbours We thus see that the longer the chain on either side of the primary effect the more the consequences are distributed and damped, and the more nearly the rate of recovery of *C* will approach its maximum, which is represented by the case when *B* and *D* do not decrease at all

A somewhat similar aspect is revealed when we consider the effect of a change in the rate constants This may be due either to a substitution (allele), or to a change in conditions, say pH, which affects the efficiency of the catalyst While the disturbance at *C* discussed above was an example of complete buffering (restoring of the steady state values), the change in the value of a rate constant permanently affects the parameters of the system Depending on the position of the constant in the system some members' s s values are affected, while others' are invariant, although they will suffer a temporary change This has already been discussed in a previous section (cf eq (10)) We can now see how the number of members affects the change in s s values due to a rate constant

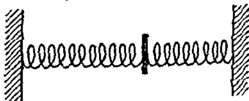
change On inspecting eq (10) it is seen that the number of members in the sequence determines the number of terms of the s s value of the first component The larger the number of members therefore, the smaller will be the effect of a change in any one step The degree of buffering is therefore increased Although subsequent members have successively one term less in their s s values, and are therefore more sensitive to changes they gain in their overall buffering behaviour by having successively one step more towards which they are completely independent In general then, the larger the number of members, the less sensitive will the components of the system be towards changes of external or internal parameters There is safety in numbers

Developmental buffering

While both the time-lag and degree of buffering are thus seen to be functions of number of members, there is another mechanism which affects time-lag without change in number The s s value of eq (10) (of which the first component is again given)

$$\bar{A} = Q_X\left(\frac{K_{AB}K_{BC}K_{CD}}{k_Y}+\frac{K_{AB}K_{BC}}{k_{CD}}+\frac{K_{AB}}{k_{BC}}+\frac{1}{k_{AB}}\right)$$

shows that the value of $\bar{A}$ can remain constant if all the rate constants are increased by the same factor as Qx is decreased (and vice versa) The difference in response of two such systems to a disturbance is best illustrated by a mechanical analogy Consider two spiral springs fixed so that they are compressed and act, via a disc, end to end in opposite directions



Depending on their respective Young's moduli the plane separating their contact ends will have an equilibrium position If the disc is displaced in one direction and then released it will begin to oscillate back and forth until it settles down again to the

original position If the springs are light and friction small the time-lag may be considerable By replacing the springs by two heavy ones (such that the equilibrium position is unaffected), the same displacement will now result in a very rapid return to the original position

Systems identical in number of members and time invariant values may therefore show considerable variation in the rate of response towards a disturbance Although this has been discussed by reference to a disturbance of the steady state of a system, it is clear that similar considerations apply to the time course of a developing system. Under a constant set of conditions, initial and persisting, the course of development is completely defined Each component (or any other function of the system) will have a path along which it moves towards the steady state If the system during its development, is subjected to random fluctuations of some of the external parameters, the actual path which any function follows will deviate from the normal to a greater or lesser extent This deviation will, apart from the extent of the fluctuation itself, depend on the 'return pressure' of the system A function which rapidly returns to the normal path may be said to possess a high degree of developmental buffering with respect to environmental fluctuations

While in the homogeneous kinetic system which serves as our model this degree of buffering is only recognised by the deviation of the actual path from the normal (the s s values being the same), in organisms many of the fluctuations are recorded in the adult phenotype The reason for this has already been indicated in the discussion on phenocopies It is essentially due to the fact that a number of effectively irreversible processes take place during specific stages in development, and these are dependent on the value of certain parameters during that time A fluctuation which affects these parameters will therefore have a permanent effect, observable in the adult This will be reflected in the environmental component of the variance of the character when it is measured in a population developing in the usual insufficiently controlled (or uncontrollable) conditions

From the simple example quoted above we can thus see that

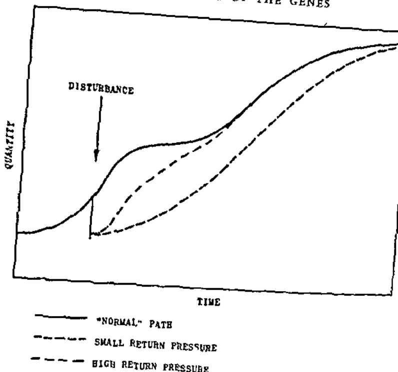


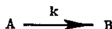
FIGURE IX
Comparison of two systems differing in their rate of response to a disturbance but reaching the same position finally

there may be two systems (which may be individuals or populations) which, though showing the same mean values, may differ considerably in their variance Whether a particular kind of buffering is of advantage (physiologically or selectively) will depend on the particular circumstance Invariance of a *certain* function may have adaptive or contra-adaptive value, and the demonstration that many responses appear to be adaptive *is* strong evidence that selection has operated to produce systems that will so respond Cases where a particular response is contra-adaptive have been much less studied, partly, perhaps, because *the* results appear to give less information than adaptive cases

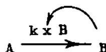
The second major factor affecting the buffering in the system (or, more generally, affecting the response characteristics) *is* complexity. This is very difficult to define in general terms (and *indeed*

may have little meaning outside particular cases) It is clearly connected with number of components, since they determine the number of ways of arranging the system. It is also connected with the number of feed-back sequences, or 'loops', which exist By introducing a new loop, the system is made more complex by the fact that this creates new interactions between components With each new interaction the response characteristic of the system is altered It is, however, not true to say that all new feed-backs tend to increase the buffering This is obvious when the nature of an autocatalytic reaction is considered

Take a simple reaction

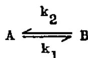


where the quantity of *A* is considered to be very large The system has no feed-back and the quantity of *B* produced is independent of the quantity already present If we now introduce a feed-back such that the quantity of *B* determines the *rate* at which *B* is being produced,



we see that instead of tending to limit the quantity of *B* it increases its rate exponentially

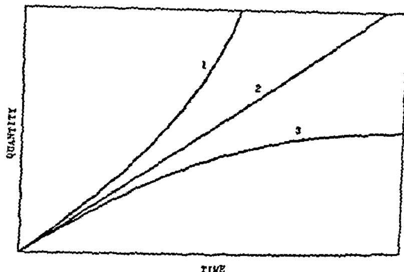
However, the introduction of a feed-back affecting the *quantity* of *B* will result in a buffering of *B*



These relations are shown in Fig. X.

Temperature buffering

While in the examples treated above, we have been concerned with disturbances and re-establishments of conditions which

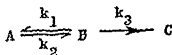


1. CONTRA-BUFFERED
2. NON-BUFFERED
3. BUFFERED

FIGURE X

operate through a mass effect, there are other factors affecting the behaviour of a system towards which invariance is found. One of the most important is temperature We shall here not be concerned with the elaborate mechanisms operating in higher animals such as the alterations in blood vessels and the evaporation of water from surfaces, by means of which the heat balance tends to be adjusted In many lower organisms, no such systems operate, and yet it is probably important that the organism should show a certain amount of buffering towards changes in temperature Below we shall give a possible mechanism operating on a chemical level, which tends to reduce the apparent temperature coefficient of a reaction and may reduce it to zero

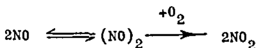
Consider the system



$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \xrightarrow{k_3} C$$

where the conversion of A to C is measured At any given temperature the rate of production of C will depend on the concentration

of *B* present If it is assumed that the absolute values of the rate constants k_1 and k_2 are larger than k_3, we are effectively measuring the latter rate constant The temperature variation of k_3 will, as usual be determined by the activation energy for this step, and hence the increase in temperature will result in an appropriate increase in the value of k_3 However, if the equilibrium constant *K* of the *A* to *B* reaction has a negative temperature coefficient, i e if the heat of reaction ΔH is positive, the concentration of *B* will decrease with a rise in temperature, thus tending to oppose the effect of the increase in the rate constant k_3 It is clear that with certain relative values of *K* and k_3, the nett temperature coefficient of the change *A* to *C* is zero, and this would constitute an effective buffering of this step towards temperature fluctuations It is of course, possible that the mass effect outweighs the rate effect, in which case an apparent negative activation energy will be found It is not uncommon to find such apparent violations of the stability conditions of a reaction when a very complex set of reactions determining a final character is studied in relation to temperature The only case which has been investigated in detail is not in biological material, but concerns the reaction



$$2NO \rightleftharpoons (NO)_2 \xrightarrow{+O_2} 2NO_2$$

where a negative temperature coefficient is in fact found

THE HIERARCHY OF THE CATALYSTS

The discussion so far has been concerned with the fate of the system's components under the influence of a certain complement of catalysts These, both as regards quantity and nature, were regarded as fixed and present in the initial state of the system These assumptions are not in accordance with the observations that in living organisms certain enzymes make their appearance at various stages of development, or change in quantity throughout life, and that enzymes are unstable to varying degrees and therefore must be continuously synthesised In any case, the single cell

from which the organism starts could *not possibly contain* a *sufficient* quantity of enzymes to keep the adult going

To deal with this problem we must introduce an aspect of molecular organisation not so far mentioned. The s s. was characterised by the time invariant values of the components, and thus the system showed a persistence of pattern in spite of the transitory nature of *many of its parts* *There is, however, another type of* persistence which relies on a different mechanism An assembly of atoms organised into a particular molecular compound may be very far removed from the lowest state of free energy which the assembly could assume It will therefore tend towards this state The rate at which it approaches this minimum, however, is determined by the free energy of activation for the transition to that state It has to go over a 'free energy hump' before it can fall into the 'trough' Part of this free energy of activation depends on the force required to break the bonds holding the atoms together The larger the number of bonds holding the structure together the greater will be the stability of the assembly We know that many macromolecules occurring in organisms (proteins nucleic acids, polysaccharides) consist of a network of interlinked units We also know that they are far from their equilibrium state since appropriate catalysts easily break up the structures Their persistence therefore depends on the extremely slow rate at which they change, and not on their being constantly maintained in that state It is persistence of pattern through stability

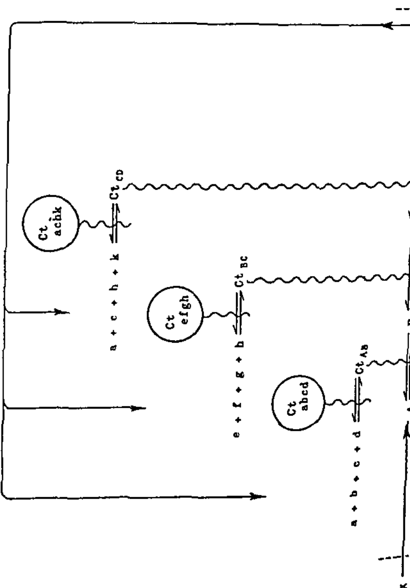
Since such stable compounds do not stand in a dynamic relation *to other compounds, their inclusion in a system* would not appear to affect its kinetics However, they interact with the components in a different way, namely through their surfaces When a compound exceeds a certain size (or *size range*) compared with molecules of 'normal' size it may gain the property of adsorbing these smaller units This is a reflection of the restricted motion of its parts and the possible co-operative *effect of neighbouring parts* The effect of adsorption on the small adsorbed molecules is to alter their reactivity In general it results in a lowering of the heat of activation, and therefore increases the *rate of reaction* of the adsorbed molecule The adsorbent acts therefore as a catalyst.

This thermodynamic basis of enzyme action forms a very important aspect of a physico-chemical interpretation of living processes The rigorous treatment of the problem, however, is beyond the scope of this discussion We shall therefore only use the fact that adsorption may result in catalysis In view of the nature of the forces involved (mainly short range coulombic and van der Waals forces) adsorption on an organic surface will be more or less specific The surface configuration will determine which molecular species can approach sufficiently closely to result in adsorption

The addition of stable and surface active components to a system therefore has the effect of altering certain of the rate constants They do not appear in the form of 'dynamic components' A, B, C etc, but affect the values of the k's The catalytic situation now assumes a more material form, since it can be specified by the quantity of particular molecular species, i e the catalysts Apart from the presence in the initial state of specific catalysts for the system's components, other surface active species may be present Their function can be the catalysis of the components which act as the catalysts which we have already considered The raw materials for the assembly of these catalysts may be a separate pool, so that we would have two parallel systems connected by the fact that some of the products of one act as catalysts for the other If we designate the catalysts by Ct with the components for which they are specific as subscripts, the formal scheme shown in diagram on page 238 can be given:

There is a hierarchy of catalysts, two classes of which are indicated The ringed class of catalysts act on a multimolecular 'assembly', reaction while the unringed class act on monomolecular 'transformation' reactions There is however no fundamental difference between them, the latter having been chosen for algebraic convenience in describing a system of first order reactions

It can be seen that only the 'highest' class of catalysts need be present in the system, since the 'lower' classes can be produced with their help Thus the lower catalysts need not have a very high stability, since they may be constantly replaced The concentrations of these catalysts may vary with time, since they are


Ct achk
a + c + h + k
Ct CD
Ct efgh
e + f + g + h
Ct BC
Ct abcd
a + b + c + d
Ct AB
x

dependent on the kinetics of their own formation and decay The kinetics of the main system may therefore be very complex.

The system as shown eventually will cease all activity when the catalyst pool is exhausted and the catalysts have decayed This is, however, not necessary, since the catalysts' raw material may be obtained from the reactions of the lowest systems, i e. by a feed-back system. The eventual source of these is of course the material which flows into the system (of which X is an example) These materials are transformed, combined and assembled in various ways to give catalysts and substrates of diverse kinds Some will have temporary existence, some will reach steady states or steady rates of production, some will fluctuate with the environment, some will be invariant Although the initial substrates and catalysts will determine the early course of development, the presence of the highest class of catalysts will determine the behaviour of the system more and more As substrates for their action become available, the catalysts so produced will feed back on the system and will begin to control it Catalysts which were not present in the beginning can thus make their appearance and open up new channels of reactions

The genes

It is now apparent that we may provisionally identify the highest class of catalysts with the genes Leaving aside for the moment the problem of their duplication, it can be seen that, even with a fixed quantity of them, neither the amount nor the nature of other catalysts is limited to that of the initial state Both the substrate system and the catalyst system can thus expand and change in a manner unpredictable from a knowledge of their initial quantities only Development of the system is therefore epigenetic The expansion is, however, controlled by the nature and quantity of the genes Its course is determined by the interaction of the pattern of the genes with the pattern of the initial state and its subsequent changes In cybernetic terminology we may say that the information in the system (of which a large part is contained in the genes) acts on the environment which therefore changes strictly in accordance with that information. In that sense development is preformationist

The genes were equated with the highest class of catalysts As such they must display an *exceptional stability, since their pattern* cannot be produced by another set of reactions For if this were so, this set of reactions would have to be catalysed by a structure having *all* the properties of a gene And so we would have an *infinite* regress, or must stop at some point and assert highest stability for it We choose to stop at the genes Their stability is therefore high but not absolute

A simple structure has a small finite number of states which its parts can take up When left to itself it will *spontaneously reach* the state of lowest free energy, though not necessarily by a direct descending route Occasionally it will jump into a higher state, but on the average its route is downwards A very complex structure does not differ from this except that the number of states is very much larger It is therefore not possible to predict to which state it is likely to change, though the direction of a large number of changes can be asserted with certainty The complex catalysts which we have called genes will therefore *occasionally* switch into another state If that new state affects the surface configuration the change will be reflected in the catalytic action it induces It may become a more efficient or a less efficient catalyst, or it may change its specificity This change will in turn be reflected in the system's properties, though some changes may be buffered out As a result the system may be better or worse from a survival or selection point of view, but this is unrelated to the change of efficiency of the catalyst or to the thermodynamic direction of the change, as has sometimes been asserted The new pattern of the structure will persist until another change brings it back or to yet a different state

We therefore see that the genes will mutate Since the *persistence* of pattern does not depend on any interaction with the rest of the system or the environment, these cannot affect it (For the obverse reasons the pattern of the dynamic part of the system cannot mutate but will be subject to environmental influences) There are, however, factors which *will influence* the rate at which mutations occur, which require special discussion.

Mutation rates

Attempts to give a unified picture of the mechanism of mutation have met with difficulties Thus the target theory based on an analysis of x-ray induced mutations is not easily related to the effects of chemical mutagens, and many thermal effects (or their absence) require special hypotheses to fit them into the scheme.

The main efforts have been directed towards elucidating *the mode of action of the agencies* responsible for the change, rather than to considerations of the *mechanism of change*

In the study of mutation rates any explanations must inevitably centre on the stability of the gene The assumption is generally made that a mutation is in the nature of a rearrangement It has often been expressed explicitly (e g McElroy and Swanson, *Quart Rev Biol*, **26** (1951), 348), and many times implied, that the stability is due to the activation energy necessary to bring the structure from one configuration to another From this it was concluded that the stable genes are those with a high energy of activation (i e sitting in low trough of energy), and the unstable genes are those having lower activation energies This is not necessarily so

The rate of transformation is determined by the *free* energy of activation If the rate is written in the formulation based on statistical thermodynamics, we find that apart from an energy (or heat) term, an entropy term is equally important in determining the rate constant

$$k = \frac{k^{+}T}{h} e^{\Delta S^{*}/R} \; e^{-\Delta H^{*}/RT}$$

This formulation implies that if a structure is to pass from a state A to a state B it must pass through a configuration C (the activated or transition state) which differs in heat and entropy from either The rate of formation of the activated state (and therefore the rate of transformation) depends on these differences in heat and entropy, ΔH^* and ΔS^*, between the normal and activated state in the manner indicated in the equation, k is the rate constant, $k^+ T/h$ is a universal frequency term and is constant for all reactions at a given T (temperature)

ΔH^* is the activation energy (or very nearly so) determinable from rate-temperature data If it is positive (as it usually is), its value will determine the 'energy hump' over which the configuration has to pass A high value will make the second exponential term a small quantity and therefore will result in a small value for k, i e the normal state A is fairly stable (and vice versa for small values of ΔH^*)

ΔS^* reflects, among other things, the change in organisation during the transition A positive value indicates that the transition state is a less ordered one, a negative value that it is more ordered than the normal state (This is only one aspect of the entropy relation, but may be taken as sufficient for the purpose of this discussion) It follows immediately that if ΔS^* has a high negative value *the rate of transformation* (*in spite of a possible low value* for ΔH^*) will be small, i e the state A is relatively stable

We therefore see that stability is not exclusively determined by the energy state of the structure, and that the *supply of additional energy, or raising of temperature, is not necessarily effective in increasing the rate of change*

One may divide reactions with high stability into three classes according to the values of the ΔH^* and ΔS^* terms, although such division is for convenience only It should be remembered that only the term

$$e^{-\Delta H^*/RT}$$

shows an appreciable variation with temperature, the other terms being temperature insensitive, at least for the range relevant to biological material A large value for ΔH^* means a large temperature coefficient of k, decreasing with ΔH^* until a value of the order of RT results in substantial temperature independence

Class	ΔS^*	ΔH^*	*Description*
1	small +ve or −ve	large +ve	This is the most common class in chemical reactions It shows varying degrees of stability depending on the actual value for ΔH^* At the same time it is temperature sensitive in the manner discussed.
2	large −ve	small +ve	This class is of similar stability to No 1 and includes some catalysed and enzyme reactions It has a small temperature coefficient

3	large −*ve*	large +*ve*	This is the most stable class and for very large values the rate is substantially zero Its rate cannot be measured but as all classes shade into one another, there is no doubt of its reality

There is no *a priori* or empirical reason to suggest that genes are more likely to belong to one of these three classes than to another We may therefore assume that, with the large number of genes usually assumed present in organisms, we may expect all three classes (and their intermediates) to be represented As far as the mechanism of change is concerned the three classes differ significantly in their behaviour towards experimental conditions

Temperature An increase in temperature will produce an increase in the rate of reaction depending on the value of ΔH^* Temperature-rate studies will therefore influence and 'detect' only those genes which are stabilised by a high activation energy, i e mainly class 1 This class will of course show a spontaneous rate since the thermal fluctuations will, from time to time, result in an accumulation of the requisite energy in the appropriate region Structures of class 2 and 3 however, may pass many times through states of the requisite energy without undergoing change They are substantially independent of their environment, at least as far as temperature or any other energy yielding process is concerned They will mutate spontaneously, their rate depending essentially on the probability of the structure passing through a particular configuration

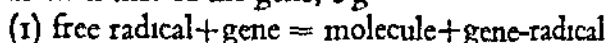
Radiations The precise mechanism of radiation induced or increased mutations is not known In the case of U V it is possible that at least part of the effects are due to an increase in energy, especially since many structures identified in or near the genes possess strong adsorption bands in the U V It is further possible that this may result in localised heating i e those regions of high adsorption receiving more energy before it is dissipated throughout the structure Such a mechanism might favour particular loci, and would result in a different mutation pattern than the normal (spontaneous) pattern which may result from equipartition of energy among all loci

There is, however, evidence that part of the action of U V and particularly that of x-rays proceeds via an indirect mechanism. It is generally held that this is through the production of secondary

electrons or such substances as peroxides, ions or free radicals Investigations of the effect of radiations on systems of known constitution show that such processes do take place

The view that these agents act as carriers of energy, giving it up as they react with gene, is, however, unlikely to be correct Such energy chains are very doubtful in solution reactions, and would certainly be rapidly deactivated by the solvent molecules of the solution.

A possible mechanism is that these agents act by exchanging their status with that of the gene, e g

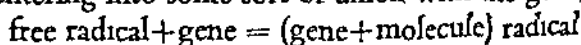


(1) free radical + gene = molecule + gene-radical

or (2) ion + gene = molecule + gene-ion.

Alternatively, production of particular substances *may only be* incidental, and the effective action may be through the direct ionisation or free radical formation of the gene In both cases such a change will in fact constitute a change in structural characteristic (with consequent changes in the constants of the rate equation), a *phenomenon which is well known in many chemical* systems In such cases transformations via the ionic or radical state may give measurable spontaneous rates, while for the normal mechanism the reaction is extremely slow The observed dose-rate relationship would still hold since the amount of measured ionisation would be *proportional to the amount of effective* gene-ionisation

On the other hand it is possible that these agents act catalytically by entering into some sort of union with the gene, e g



free radical + gene = (gene + molecule) radical

Any substance which enters into some relationship with a system will alter the structural and energetic properties Catalysts and enzymes are known to act by changing the constants ΔH^* and ΔS^* in a way favourable to the rate, and we may extend the term catalyst to describe any substance which, by *entering into* some relation with another changes its rate parameters

Chemical mutagens Their mode of action is equally uncertain. It is more likely that the *catalytic mechanism* suggested above applies to this case The mutagenic agents themselves, *or their* decomposition or reaction products, *may by interaction* with specified groupings produce conditions more favourable to the mechanism of change Such changes in the rate constants can

occur in all three classes considered, but certain structures will be more readily affected than others This means that the radiation or chemically induced mutation pattern will not necessarily be the same as the temperature and other patterns

We may sum up the situation as follows

(1) The population of genes constituting the genotype of the organism can be arranged in a spectrum as far as the factors ΔH^* and ΔS^* affecting their stability are concerned

(2) Owing to the difference in these parameters any particular mutagenic agency will, in general, affect a different portion of the spectrum There will be an overlap, but each agency will cause the appearance of a particular mutation pattern for the organism characteristic of the mode of action of the agency

(3) Conclusions drawn from the study of one of these agents are not necessarily relevant to the mechanism of the others

(4) The same dose of a given mutagen should in general affect the mutation rate of two loci to a different extent

(5) The mutagenic pattern of a given gene, i e the effect of a series of agents on the rate, will be characteristic of that gene, and might be used as a means of distinguishing whether two separately arisen 'identical' mutations are in fact so

These conclusions are in accord with the results obtained in *Neurospora* (Kolmark, Hereditas XXXIX (1953), 270) and in *E coli* (Demerec, Proc 9th Internat Congr of Genetics (1954), 201)

Inter-genic relations

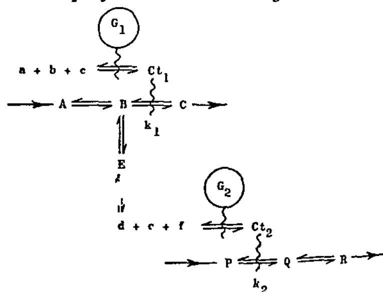
So far we have treated the genes as if they were separate macromolecular entities with a specific surface controlling a specific catalyst This appears to suggest a 'one gene—one enzyme' relationship The situation is, however, more complex than this We have seen that multiple effects can be caused by the change in a single rate constant Insofar as each enzyme is assumed to control a single reaction—'one enzyme—one rate constant'—demonstration of primary pleiotropic effects on reaction *products* does not invalidate the 'one gene—one enzyme' hypothesis Thus was the state of affairs in the simple systems described at the beginning Since then we introduced the idea of a feed-back of reaction products

to the gene-system This alters the relations in an important way

In all our discussions on substitution effects we compared two systems which differed in the magnitude of one (or more) rate constants This change in the magnitude of k may be due to a variety of causes if it is controlled by enzymes

Let us assume we have two systems which differ—by mutation or substitution—in a single gene By this we mean that the two surface configurations of one 'highest class catalyst' differ in some material way The catalysts derived from these will therefore also show a changed surface configuration Consequently the adsorption parameters for the relevant metabolic molecules will differ and the two catalysts will cause different values of rate constants k_1 and k_1*, to operate in the two systems These rate constants enter the expression of many components of the system Some of these components will be molecules participating in some catalysogenic reactions If for example the substitution of k_1* results in a [illegible] con-

[illegible] of this second catalyst will be present in [illegible] has the effect of decreasing the magnitude of the dependent rate constant from k_2 to k_2* The situation is shown diagrammatically below



The genetic change occurs from G_1 to G_1^* and thus can be seen to have an effect on the value of *two* rate constants On k_1 by a *qualitative* change of Ct_1 to Ct_1^* and on k_2 by a *quantitative* change of nCt_2 to mCt_2 We therefore have gene interaction 'one gene—two enzymes' It is clear that much more complex interactions can occur in the organism

The catalytic efficiency of enzymes is known to be highly dependent on the pH of the medium The pH, like other characters is under genetic control (cf the flower pigments in *Primula*) A particular genetic change may therefore affect a whole class of enzymes insofar as it affects the medium pH and alters the resulting rate constants 'one gene—many enzymes' We may therefore conclude that the method of eliminating pleiotropic effects by measuring rate constants will not necessarily establish whether one or more enzymes are *directly* affected

The picture of the gene developed so far does not restrict it to an existence as a separate physical entity The macro-molecules can be combined into larger structures and there is good evidence that they are The mere aggregation of the genes would not alter the arguments advanced so far Since however the activity of the gene was considered to be vested in its surface the juxtaposition of two genes can have effects on the surface specificity The distribution of van der Waals forces from point to point is a co-operative effect of all the atoms in the neighbourhood This effect decreases with distance but its precise distribution is difficult to assess The nature of the units which a gene assembles in producing directly or indirectly an enzyme may therefore be affected by the genes adjacent to it We therefore would find a 'position effect' of enzyme surface structure which would be reflected in the phenotype

The assumption of a large structure with a continuous surface raises the question what precisely an individual gene is Although any particular portion of the surface has lost much of its identity it will still act by eventually determining the specificity of the catalytically active enzyme surface This in turn will control the rate of a reaction The conversion of one single molecular species into another constitutes the individuality of the process Alteration

of the genetic surface may affect the rate and final concentrations, but the identity of the molecules which are acted upon will not be influenced It is hardly fair to call this the 'death of the gene' Although it may be difficult to define where it begins and ends, the specificity of its action and the power of its effect remain very much alive

Structural changes of portions of the surface can occur in the same manner as those described for individual macro-molecules In addition a structural change may take place in the region 'between' two genes, i e between two portions carrying the *specificity of particular enzymes Such a change may therefore* affect both genes In such a case we may write

One mutation—two genes
Two genes—several enzymes
Several enzymes—many rate constants
Many rate constants—a multitude of reactions

In view of this one may wonder how Mendel could have laid the foundations of genetics Fortunately for him and later investigators most of the effects will be eliminated by buffering and, of the remaining, many will not be apparent by inspection In general, many mutations must occur which have no apparent effects In *consequence, the mutation rate of organisms may* be very much higher than the estimates indicate, since the wild type and the mutant may mutate many times to alleles substantially identical in phenotype (It may also be questioned how long *isogenic* stocks will remain so after cessation of inbreeding)

Apart from showing catalytic properties which control the development of the organism the genes are capable of self-duplication Put more correctly, the genetic material is continuously being reproduced in the course of cell division It is not useful to describe the genes as 'autocatalytic', as an analysis of any autocatalytic chemical reaction shows Such processes are the breakdown of a complex structure catalysed by one of the breakdown products These products are therefore already contained in the structure and the reaction merely liberates them. Gene reproduction, on the contrary, involves the creation of identical units from unorganised material It is not the property of any one

substance or particle but the property of a system (cf Kacser, *Science*, **124** (1956) 151) It appears that the process is intimately connected with the fact that the genes are organised into larger units, the chromosomes or structures equivalent to them Since this is a problem about which much has been written elsewhere it will not be discussed here

CONCLUSION

It will by now be obvious to the reader that, given ingenuity and patience, a large number of kinetic systems can be constructed and their properties compared with biological phenomena Some of these schemes may have immediate heuristic value while others would, in the present state of knowledge, provide conceptual models only It has often been argued that these latter are valueless, and indeed if they are offered as 'explanations' this criticism is not without foundation There is, however, one aspect of physico-chemical models which sets them apart from other models Biology and physical chemistry are in many ways closely related They are both concerned with the analysis of process and mechanism If we believe that the biological process is the result of an underlying physico-chemical one, then a re-statement of the problem in these terms appears the most appropriate Where there is no immediate prospect of experimental verification of a model, it may, nevertheless, not be without value The habit of thought—to give an interpretation in terms of concepts successfully applied in an exact science—will, it is hoped, lead to a re-orientation of experimental approaches The newer methods and techniques which today are so successfully applied in many fields of biology can become more than better tools In a sense they can create new problems, and allow us to ask more searching questions to which our curiosity demands an answer

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